DUE IN STATION LIBRARY JUL 14 1953

Volume 33 MAY-JUNE 1953 No. 3

DUE IN STATION LIBRARY JUL 22 1953

CANADIAN JOURNAL OF AGRICULTURAL SCIENCE

EXPERIMENT STATION LIBRARY

(formerly *Scientific Agriculture*)

DUE IN STATION LIBRARY AUG 20 1953

DUE IN STATION LIBRARY AUG 27 1953

JUN 30 1953 PURDUE UNIVERSITY

CONTENTS

Growth depressing factors in rapeseed oilmeal *J. M. Bell and K. Williams* 201

The effect of various canning crop rotations on certain soil constituents over a five-year period *H. Hill, R. F. Bishop and H. B. Cannon* 210

Observations on the mineral metabolism of pullets. X. The effects of gonadal hormones on retention and turnover of calcium by the skeleton *J. R. Jowsey, W. F. Oliver, W. A. Maw and R. H. Common* 216

A study of soil and fertilizer phosphorus uptake on a calcareous soil by oat plants, using radioactive tracer technique *D. D. Dolson and J. W. Ketcheson* 225

Consumptive use of water in orchard soils. II. Effects of evaporating power of air and of length of irrigation interval *J. C. Wilcox, J. L. Mason and J. M. McDougald* 231

A study of the carotene and crude protein content of orchardgrass (*Dactylis glomerata* L.) II. Heritable variation - *M. F. Clarke* 246

Molybdenum poisoning of cattle in the Swan River Valley of Manitoba - *Hugh M. Cunningham, J. M. Brown and Arnold E. Edie* 254

The relationship between 30-, 60-, 90- and 120-day non-returns to service in the artificial insemination of dairy cattle in Ontario - - - - - - - *T. D. Burgess* 261

Water retention and availability in soils related to drought resistance - - - *J. J. Lehane and W. J. Staple* 265

Studies on the effect of light on the growth and development of red clover - - *R. A. Ludwig, H. G. Barrales and H. Steppler* 274

Preliminary report on the effect of spray for corn borer control on yields of field corn - - *G. F. H. Buckley and C. G. Mortimore* 288

A study of the inter-relationships of seed yield, oil content, and other agronomic characters with sunflower inbred lines and their top crosses - - - - *W. A. Russell* 291

EDITED BY THE
AGRICULTURAL INSTITUTE OF CANADA
OTTAWA 4, CANADA

EDITORIAL BOARD

R. H. COMMON, Professor and Chairman, Department of Chemistry, Macdonald College, Que.—*Chairman.*

J. F. BOOTH, Chief, Economics Division, Marketing Service, Canada Department of Agriculture, Ottawa, Canada.

W. H. COOK, Director, Division of Applied Biology, National Research Council, Ottawa, Canada.

E. W. CRAMPTON, Professor and Chairman, Department of Nutrition, Macdonald College, Que.

L. W. J. HURD, General Secretary, Agricultural Institute of Canada, Ottawa, Canada.

T. JOHNSON, Plant Pathologist, Dominion Laboratory of Plant Pathology, Winnipeg, Man.

A. G. McCALLA, Dean, Faculty of Agriculture, University of Alberta, Edmonton, Alta.

G. N. RUHNKE, Research Director, Ontario Department of Agriculture, c/o O.A.C., Guelph, Ont.

H. L. TRUEMAN, Director, Administrative Services, Canada Department of Agriculture, Ottawa, Canada.

J. C. WOODWARD, Chief, Division of Chemistry, Science Service, Canada Department of Agriculture, Ottawa, Canada.

Managing Editor - - - L. W. J. HURD
Editor - - - - HILDA GRAY

Published monthly by the authority of the Chairman of the National Committee on Agricultural Services

NATIONAL COMMITTEE ON AGRICULTURAL SERVICES

Chairman

RT. HON. JAMES G. GARDINER, Minister of Agriculture for Canada

Provincial Ministers of Agriculture

Hon. KENNETH KIERNAN - - - Victoria, British Columbia
Hon. D. URE - - - - - Edmonton, Alberta
Hon. I. C. NOLLET - - - - Regina, Saskatchewan
Hon. R. D. ROBERTSON - - - Winnipeg, Manitoba
Hon. FLETCHER THOMAS - - - Toronto, Ontario
Hon. L. BARRÉ - - - - Quebec, Quebec
Hon. C. B. SHERWOOD - - - Fredericton, New Brunswick
Hon. A. W. MACKENZIE - - - Halifax, Nova Scotia
Hon. C. C. BAKER - - - - Charlottetown, Prince Edward Island
Hon. F. W. ROWE - - - - St. John's, Newfoundland

The National Committee on Agricultural Services functions as a co-ordinating medium on agricultural services and agricultural policy in Canada, and has as an advisory body the group of technical officers listed on the back cover.

CORRESPONDENCE AND SUBSCRIPTIONS

Address all correspondence to the Agricultural Institute of Canada, Grindley Hall, 338 Somerset Street West, Ottawa 4, Canada. *Canadian Journal of Agricultural Science,* (formerly *Scientific Agriculture*) will be mailed to any address in Canada, the United States, Mexico, Central and South America and the British Commonwealth for $3.00 a year; other countries $4.00 a year. Single copies, 50 cents.

Authorized as second class mail, Post Office Department, Ottawa.

CANADIAN JOURNAL OF AGRICULTURAL SCIENCE

(*formerly* Scientific Agriculture)

VOLUME 33 MAY-JUNE, 1953 No. 3

GROWTH DEPRESSING FACTORS IN RAPESEED OILMEAL[1]

J. M. BELL[2] AND K. WILLIAMS[3]

University of Saskatchewan, Saskatoon, Sask.

[Received for publication October 14, 1952]

ABSTRACT

Rapeseed oilmeal contains a water-soluble growth depressing factor. Growth rates in mice comparable to those obtained on linseed oilmeal-supplemented rations were possible when the amount of rapeseed oilmeal was limited to 18 per cent, or when 0.5 per cent APF was added to the ration. No practical method of directly counteracting the action of the factor was found. Iodinated casein (0.1 per cent) was ineffective in rations containing up to 27 per cent rapeseed oilmeal, although it has been reported to be effective for fowl and rats.

These experiments suggest either species differences in response to the rapeseed goitrogen or the presence of more than one deleterious factor in rapeseed oilmeal.

INTRODUCTION

Certain characteristics of rapeseed oilmeal have imposed definite limitations on its use in livestock and poultry feeding. It is rather unpalatable to some animal species and the *Brassica* genus has been shown to possess a goitrogenic factor, 1-5-vinyl-2-thiooxazolidone (2, 3, 4, 6, 7, 9, 17, 21, 24). A number of methods have been proposed to permit more extensive use of the oilmeal, among which are limiting the level used to 10 or 20 per cent of the ration (6, 11, 20); adding iodinated casein (7, 21), or inorganic iodide (1, 17) to prevent thyroid hyperplasia. Water extraction (3) and treatment with steam (14) also proved beneficial. However, apparent inconsistencies in results obtained and the serious lack of information pertaining to the use of rapeseed oilmeal in large animal feeding underline the importance of further research, with particular emphasis on the basic problems involved.

Many of the nutritional investigations involving *Brassica* have been concerned primarily with thyroid dysfunction as measured by degree of thyroid enlargement whereas in practice this approach is satisfactory only in so far as thyroid hyperplasia may be correlated with depressed growth or production. Some goitrogenic substances, like thiouracil and thiourea, have potential value in animal feeding.

In the experiments reported here an attempt has been made to confirm some of the published findings regarding tolerable levels of rapeseed oilmeal,

[1] Contributed by Department of Animal Husbandry, University of Saskatchewan, Saskatoon, Sask.
[2] Associate Professor of Animal Husbandry.
[3] Student assistant; now with Canada Department of Agriculture, Summerland, B.C.

water extraction, and iodinated casein. In addition the efficacies of antibiotics and vitamin B_{12} were assessed. Antibiotics were included because of the report (3) that thiooxazolidone was apparently released by enzyme action from a precursor. Since contained enzymes would presumably be inactivated by the heat developed in normal expeller-processing it was possible that intestinal microflora might elaborate appropriate enzymes and thus antibiotics might curtail the release of the goitrogen. Vitamin B_{12} was included because it does not abound in plant material (16, 18) and has been variously involved in thyroid activity (5, 8, 10, 12, 19, 22, 23).

MATERIALS AND METHODS

Animals and management

Mice of Carworth Farms No. 1 strain, several generations of which have been raised in this laboratory, were used in these experiments. When 21 days of age weanlings were allotted at random to diets and to individual cages of a battery in a thermostatically controlled room. Weight gains were recorded over a 2-week period since this embraces the interval of most rapid post-weaning growth in the normal mouse. Feed and water were provided *ad libitum*.

Experiment No. 1. This was primarily an attempt to confirm with mice the general recommendations formulated for turkey poults and poultry. Levels of rapeseed oilmeal ranging up to 27 per cent in diets containing 20 per cent protein were used. As sub-treatments in a factorial design, tests were also made of 0.1 per cent iodinated casein (Protamone[1]), of vitamin B_{12} at 50 μg./kg. diet, and of 0.1 per cent propyl-thiouracil. Both male and female mice were employed, two of each sex per diet.

The formulas for the five basal diets employed are listed in Table 1 and the method of supplementation is illustrated in Figure 1.

TABLE 1.—FORMULAS OF BASAL DIETS CONTAINING RAPESEED OILMEAL OR WATER-EXTRACTED RAPESEED OILMEAL

Ingredient	Percentage rapeseed oilmeal				Water-extracted rapeseed oilmeal
	27	18	9	0	
	%	%	%	%	%
Wheat	29	29	29	29	34
Oat groats	25	25	25	25	25
Wheat bran	10	10	10	10	10
Vita grass	5	5	5	5	5
Linseed oilmeal	—	9	18	27	—
Rapeseed oilmeal	27	18	9	—	—
Ext. rapeseed oilmeal	—	—	—	—	22
Bone meal	2	2	2	2	2
Salt, iodized	1	1	1	1	1
Fish oil (2000A, 400D)	1	1	1	1	1
Riboflavin, mg/kg.	4	4	4	4	4
$MnSO_4$, mg/kg.	22	22	22	22	22

[1] Courtesy Cerophyll Laboratories Inc., Kansas City, Mo.

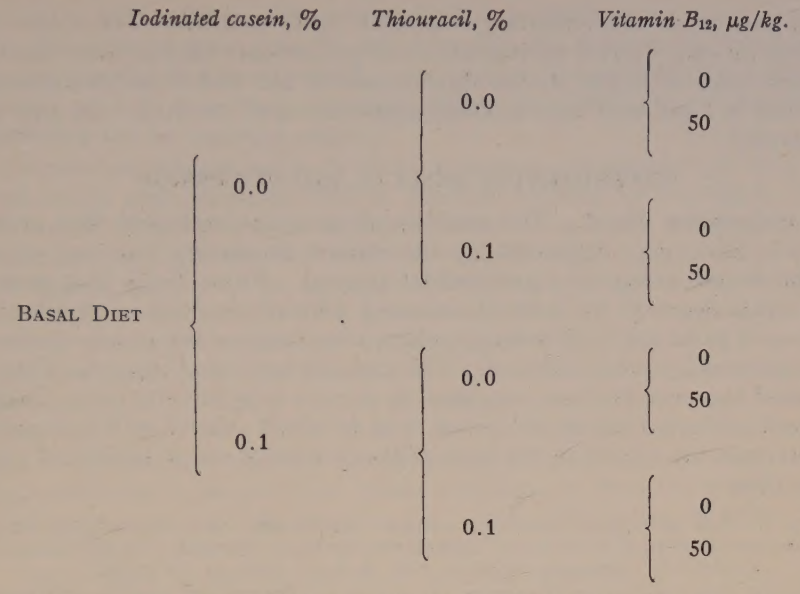


FIGURE 1. Diagram illustrating the various combinations of iodinated casein, thiouracil and vitamin B_{12} added to each of the five basal rations in Experiment 1.

Experiment No. 2. The object of this study was to obtain data on the responses of male and female mice to various levels of thiouracil because sex differences became evident in the first trials. The basal diet used in this case was composed of wheat 65, alfalfa leaf meal 5, skim-milk powder 27, mineral mixture 3 per cent and supplementary vitamins A, D and E. The levels of thiouracil were 0.00, 0.01, 0.02, 0.04, 0.08, 0.16, and 0.32 per cent of the diet and, as before, two male and two female weanlings were fed for a 14-day assay period and gains were recorded.

Experiment No. 3. This was a study of the value of flavouring agents for improving growth response through increased feed consumption. The level of rapeseed oilmeal was increased over that used in the first experiment in order that comparisons between rapeseed oilmeal and linseed oilmeal would not reflect differences in amino acid assortment (protein quality) at this stage. The amount of thiouracil used was correspondingly increased (to 0.2 per cent) and was included again to obtain further data on the similarity of this product with the factor in rapeseed oilmeal.

Two antibiotics were tested. APF[2] and ustilagic acid (crystalline)[3] (13) were added at levels of 0.5 and 0.05 per cent of the diet respectively. The experimental design was similar to that used in the first experiment (Figure 1). The APF thus provided about 2 p.p.m. penicillin and 20 µg./kg. diet of vitamin B_{12}, and the ustilagic acid level was 500 p.p.m. in the diet.

The basal diet formula was wheat 42.8, alfalfa leaf meal 2.0, rapeseed oilmeal or linseed oilmeal 51.2, dried brewers' yeast 1.0, mineral mixture[4]

[2] Animal Protein Factor supplement No. 3. Courtesy Merck & Co. Ltd., Montreal, Que.
[3] Courtesy R. H. Haskins, Prairie Regional Laboratory, National Research Council, Saskatoon, Sask.
[4] Bone Meal 280, ground limestone 470, NaCl 250, KI-calcium stearate 0.2, $FeSO_4$ 20, $MgSO_4$ 20, $MnSO_4$ 0.5 and $CuSO_4$ 0.1 gm.

3 per cent and supplementary vitamins A, D and E[5]. The diets containing linseed oilmeal served as a control series. Of the four rapeseed oilmeal basal diets one had no flavour added, one had 2 per cent sucrose, one had 0.2 per cent monosodium glutamate and one had 0.04 per cent fenugreek[6].

EXPERIMENTAL RESULTS AND DISCUSSION

Experiment No. 1. The analysis of the gains indicated that growth rates in mice were depressed by the rations employing rapeseed oilmeal as the major source of supplemental protein. From Table 2 it appears that each decrease in level of rapeseed oilmeal resulted in significantly improved gains until the instance where two-thirds of the rapeseed oilmeal was replaced by linseed oilmeal. The variance analysis (Appendix Table 1) revealed however that the responses to protein supplements were modified by both iodinated casein and sex of mice involved; therefore it is necessary to interpret the results on the basis of the protein × sex × iodinated casein interaction.

TABLE 2.—THE EFFECTS OF IODINATED CASEIN, THIOURACIL, AND SEX OF MICE ON THE GROWTH RESPONSE TO VARIOUS LEVELS OF RAPESEED OILMEAL AND TO WATER-EXTRACTED RAPESEED OILMEAL (GM. WEIGHT GAIN IN 14 DAYS)

Treatments	Percentage rapeseed oilmeal				Water-extracted rapeseed oilmeal (22%)
	27	18	9	0	
Average gains (necessary difference = 0.7 gm.)					
All treatments	4.7	6.5	7.4	7.7	9.4
Iodinated casein × *sex* (nec. diff. = 1.4 gm.)					
No iodinated casein					
Males	6.2	8.5	8.2	8.1	8.7
Females	3.9	5.6	5.8	6.5	6.7
0.1% iodinated casein					
Males	3.7	6.0	9.1	8.0	12.6
Females	4.9	6.0	6.5	8.5	9.8
Thiouracil × *iodinated casein* (nec. diff. = 1.4 gm.)					
No iodinated casein					
No thiouracil	6.2	9.4	9.7	9.2	9.7
0.1% thiouracil	3.9	4.8	4.3	5.1	5.8
0.1% iodinated casein					
No thiouracil	5.3	6.7	8.6	8.7	11.5
0.1% thiouracil	3.3	5.1	7.1	7.7	10.9

The data also show that in the absence of iodinated casein there was no advantage in decreasing the amount of rapeseed oilmeal below 18 per cent for either sex. When iodinated casein was incorporated in the rations only

[5] Oleum percomorphum (60,000A, 8500D) 8.0 mg., alpha tocopherol 500 mg., and corn oil to 100 ml. volume. Used 1/8 ml. per 100 mg. diet.

[6] Courtesy Flavor Corporation of America, Chicago, Ill.

the males responded to successive changes in level of rapeseed oilmeal. This behaviour appeared to be due primarily to the fact that iodinated casein markedly depressed growth in males when fed high levels of rapeseed oilmeal, as if it were accentuating, rather than alleviating, the growth depression due to rapeseed oilmeal.

These sex differences are difficult to explain adequately but they may be of major importance in feeding rapeseed oilmeal to live stock. It is improbable that the level of iodinated casein was much too high (toxic) since its use resulted in growth stimulus with extracted rapeseed oilmeal and with linseed oilmeal (with females). The results suggest that iodinated casein failed to counteract the growth depressor(s) in rapeseed oilmeal however well it may have prevented thyroid hyperplasia. The growth depression due to iodinated casein in males fed 18 and 27 per cent rapeseed oilmeal-diets may reflect normal differences in thyroxine requirements. Female mice have been shown to produce more thyroxine than males (15); thus it might be expected that the optimum levels of iodinated casein would differ according to sex and that under conditions of retarded growth males would react more markedly than females to thyroxine-active material, unless thyroxine *per se* was required to overcome the retarded growth.

Since there are several points of possible interference with thyroxine activity it need not be expected that the goitrogen in rapeseed oilmeal behaves like any of the better-known goitrogens. The results show a dissimilarity between thiouracil and rapeseed oilmeal effects. The addition of thiouracil consistently reduced gains compared to the corresponding controls and it is also evident that 0.1 per cent thiouracil when added to extracted rapeseed oilmeal produced growth depression approximating that on the 27 per cent rapeseed oilmeal diet. However, the addition of iodinated casein to rapeseed oilmeal diets was completely ineffective although it was capable of counteracting thiouracil additions to linseed oilmeal and to extracted rapeseed oilmeal diets. It therefore seems that the goitrogen in rapeseed oilmeal is either not amenable to correction by iodinated casein in the mouse or that another growth depressant besides thiooxazolidone exists in rapeseed oilmeal. That the latter is the more probable case is shown by studies to be reported elsewhere and which indicated slow growth in mice fed rapeseed oilmeal even though thyroids were not seriously enlarged.

Water extraction of the oilmeal resulted in marked improvement of the product, indicating that some water-soluble, deleterious factor(s) had been removed and confirming earlier work (3). Also of interest is the finding that extracted rapeseed oilmeal plus iodinated casein was distinctly better than linseed oilmeal. This may reflect a more favourable amino acid balance in the rapeseed oilmeal rations or the presence of some factor in linseed oilmeal which interferes with the full expression of the iodinated casein stimulus.

No definite response was obtained to vitamin B_{12} but, since one of the higher order interactions involving this vitamin proved statistically significant, it appeared to warrant further study.

In summarizing the results of this experiment it appears that up to 18 per cent rapeseed oilmeal may be used in mixed rations without incurring

serious growth depressing effects. This is in agreement with work cited earlier for other species. The use of 0.1 per cent iodinated casein was ineffective in alleviating growth depression due to rapeseed oilmeal but the actual effect on the thyroid glands was not investigated. The results confirm the water-soluble nature of a growth depressor in rapeseed oilmeal and indicate further that there may be more than one deleterious factor in this product.

Experiment 2. The results of the separate study of the effects of various levels of thiouracil on growth are presented in Table 3. Parallel responses occurred for the two sexes of mice, a relationship most apparent when gains are expressed as percentages of 'normal' gains on control animals of similar sex. It is thus difficult to explain the sex differences that occurred in Experiment 1 on the basis of thyroid function alone.

TABLE 3.—GROWTH OF MALE AND FEMALE WEANLING MICE FED DIETS CONTAINING SEVERAL LEVELS OF PROPYLTHIOURACIL

Thiouracil in diet (%)	Two-week gains		Percentage of normal growth	
	Males	Females	Males	Females
0.00	14.4	11.9	100	100
0.01	12.0	10.7	83	90
0.02	10.8	7.7	75	65
0.04	8.2	7.2	57	61
0.08	7.5	4.5	52	38
0.16	6.1	5.0	42	42
0.32	4.5	3.8	31	32

Experiment No. 3. Growth rates of mice receiving high levels of rapeseed oilmeal in their diets were not appreciably improved by the use of flavouring agents. The highest average gain was obtained when monosodium glutamate was added but the difference over the control was not statistically significant and in no case did the gains approach those made by mice consuming linseed oilmeal diets.

The use of 0.5 per cent APF resulted in gains on rapeseed oilmeal diets equalling those in mice receiving the linseed oilmeal diets without antibiotic supplements (Table 4). Similar improvement occurred, however, when APF was added to linseed oilmeal; hence there is little suggestion that APF has directly counteracted the growth depressing factor(s) in rapeseed oilmeal. The antibiotic ustilagic acid was ineffective with rapeseed oilmeal diets although it resulted in some improvement with linseed oilmeal, especially when used in conjunction with APF.

Of some interest is the finding that the use of 0.2 per cent thiouracil did not obscure responses to APF and ustilagic acid on rapeseed oilmeal diets, and, also worthy of note, the gains made on rapeseed oilmeal diets with thiouracil were equal to those on linseed oilmeal diets with thiouracil. Thus with no additive effects apparent between thiouracil and the rapeseed oilmeal growth depressant(s) there is another indication that the modes of action are different.

In conclusion, these experiments have shown rapeseed oilmeal to contain a water-soluble, growth-depressing factor (for mice). While no

TABLE 4.—RESULTS OF ADDING ANTIBIOTIC SUPPLEMENTS IN THE PRESENCE OR ABSENCE OF THIOURACIL, IN TERMS OF AVERAGE TWO-WEEK GAINS IN WEANLING MICE

Protein and antibiotic supplement used	No. of mice per group	Thiouracil		Necessary difference(1)
		Nil	0.2%	
		gm.	gm.	gm.
Rapeseed oilmeal (2)				
No APF	16	4.8	1.7	1.0
0.5% APF	16	7.4	2.7	
No ustilagic acid	16	6.0	2.3	1.0
0.05% ustilagic acid	16	6.2	3.6	
No APF, no ustilagic acid	8	4.4	1.8	1.4
0.5% APF, 0.05% ustilagic acid	8	7.2	2.7	
Linseed oilmeal—				
No APF, no ustilagic acid	2	7.5	2.0	2.8
0.5% APF, no ustilagic acid	2	9.5	2.8	
No APF, 0.05% ustilagic acid	2	9.8	3.0	
0.5% APF, 0.05% ustilagic acid	2	12.2	4.2	

(1) P = 0.05.
(2) These data represent the average of all flavour treatments since flavours did not significantly influence other responses.

practical method of directly counteracting the action of this factor was revealed, growth rates comparable to those obtained on linseed oilmeal rations were possible when the amount of rapeseed oilmeal was limited to 18 per cent, or when 0.5 per cent APF was incorporated in the ration. Palatability was not a major drawback in mixed rations nor was it appreciably improved by the addition of various flavouring agents.

Although its effect on thyroid size was not studied it was clear that 0.1 per cent iodinated casein was of no value in counteracting the growth depressor(s) in diets containing up to 27 per cent rapeseed oilmeal. Iodinated casein has been reported effective in other species (fowl, rats); hence these experiments suggest either species differences in response to the rapeseed goitrogen or, more likely, the presence of more than one deleterious factor in rapeseed oilmeal.

ACKNOWLEDGMENTS

The authors wish to express their appreciation for the assistance rendered by E. Y. Spencer, formerly of the Chemistry Department, University of Saskatchewan, now at Science Service Laboratory, Canada Department of Agriculture, London, Ontario; and by Z. Raciszewski of the Chemistry Department, University of Saskatchewan.

Financial assistance was provided by the National Research Council of Canada.

REFERENCES

1. Allen, C. E., and D. S. Dow. The biological assessment of the value of rapeseed oilmeal as a dietary component. Sci. Agr. 32 : 403–410. 1952.
2. Astwood, E. B., M. A. Greer, and M. G. Ettlinger. The antithyroid factor of yellow turnip. Science 109, 2843, 641. 1949.

3. Astwood, E. B., M. A. Greer, and M. G. Ettlinger. 1-5-vinyl-2-thiooxazolidone, an antithyroid compound from yellow turnip and from Brassica seeds. J. Biol. Chem. 181 : 121-130. 1949.
4. Bell, J. M., and J. A. Weir. Supplementation of alfalfa and marsh hays with linseed, rapeseed and mustardseed oilmeals in gestation rations for ewes. Sci. Agr. 32 : 496–501. 1952.
5. Betheil, J. J., and H. A. Lardy. Comparative effectiveness of vitamin B_{12}, whole liver substance and extracts high in APA activity, as growth promoting materials for hyperthyroid animals. J. Nutrition 37 : 495-509. 1949.
6. Blakely, R. M., and R. W. Anderson. Studies with rapeseed oilcake meal. I. The effect of various levels of rapeseed oilcake meal in the diet on the weight of the thyroid glands of turkey poults. Sci. Agr. 28 : 393–397. 1948.
7. Blakely, R. M., and R. W. Anderson. Studies with rapeseed oilcake meal. II. The effect of the inclusion of protamone in the diet, on the thyroid enlargement induced by the feeding of rapeseed oilcake meal to turkey poults. Sci. Agr. 28 : 398–402. 1948.
8. Bosshardt, D. K., W. J. Paul, K. O'Doherty, J. W. Huff, and R. H. Barnes. Mouse growth assay procedures for the "animal protein factor". J. Nutrition 37 : 21-36. 1949.
9. Burkitt, W. H. Apparent digestibility by lambs of grass hay supplemented with rapeseed oilmeal or linseed oilmeal. Mont. Agr. Exp. Sta. Circ. 193. 1951.
10. Emerson, G. A. Growth promoting activity of vitamin B_{12} in rats receiving thyroid substance. Proc. Soc. Exp. Biol. Med. 70 : 392-394. 1949.
11. Fanguaf, R., and A. Haensel. Rye bran, dried brewers' grains, malt combs and ground rapeseed cake for hens. Atch. Geflugelk. 12 : 149-158. 1938.
12. Frost, D. V., H. H. Fricke, and H. C. Spruth. Rat growth assay for vitamin B_{12}. Proc. Soc. Exp. Biol. Med. 72 : 102-106. 1949.
13. Haskins, R. H. Biochemistry of the Ustilaginales. I. Preliminary cultural studies of *Ustilago Zeae*. Can. J. Res., *C*, 28 : 213–223. 1950.
14. Hercus, C. E., and H. D. Purves. Studies on endemic and experimental goitre. J. Hyg. 36 : 183–203. 1936.
15. Hurst, V., and C. W. Turner. The thyroid secretion rate in the mouse and its relation to various physiological processes. Mo. Agr. Exp. Sta. Res. Bull. 417. 1948.
16. Ivey, J. E. Sources of vitamin B_{12} in feed. Feedstuffs 26. Aug. 1950.
17. Kennedy, T. H., and H. D. Purves. The effect of Brassica seed diets on rats. Brit. J. Exp. Path. 22 : 241-244. 1941.
18. Lewis, U. J., U. D. Register, H. T. Thompson, and C. A. Elvehjem. Distribution of vitamin B_{12} in natural materials. Proc. Soc. Exp. Biol. Med. 72 : 479. 1949.
19. Meites, J. Effects of vitamin B_{12} on thiouracil action in rats. Proc. Soc. Exp. Biol. Med. 75 : 193-195. 1950.
20. Pettit, J. H., S. J. Slinger, E. V. Evans, and F. N. Marcellus. The utilization of sunflower seed oilmeal, wheat distillers' dried grains and rapeseed oilmeal in poultry rations. Sci. Agr. 24 : 201–213. 1944.
21. Purves, H. D. Studies on experimental goitre. IV. The effect of di-iodotyrosine and thyroxine on the goitrogenic action of Brassica seeds. Brit. J. Exp. Path. 24 : 171-173. 1943.
22. Register, U. D., W. R. Ruegamer, and C. A. Elvehjem. An improved assay for a growth factor in liver extracts. J. Biol. Chem. 177 : 129. 1949.
23. Sure, B., and L. Easterling. The protective action of vitamin B_{12} against the toxicity of dl-thyroxine. J. Nutrition 42 : 221-226. 1950.
24. Turner, C. W. The effect of rapeseed on the thyroid of the chick. Poultry Sci. 25 : 186-187. 1948.

APPENDIX

TABLE 1.—ANALYSIS OF VARIANCE OF WEIGHT GAINS FROM EXPERIMENT 1

(Small variance values included in "Error")

Source	Degrees of freedom	Variance
Proteins	4	48.78**
Iodinated casein	1	9.13**
Thiouracil	1	147.44**
Vitamin B_{12}	1	.90
Sex	1	45.09**
Proteins × iod. casein	4	13.07**
Proteins × sex	4	3.59*
Iod. casein × thiouracil	1	33.87**
Iod. casein × vitamin B_{12}	1	12.04**
Iod. casein × sex	1	10.18**
Iod. casein × proteins × sex	4	3.53*
Iod. casein × proteins × thiouracil	4	2.55
Proteins × thiouracil × vitamin B_{12}	4	2.42
Error	128	1.996

TABLE 2.—ANALYSIS OF VARIANCE OF WEIGHT GAINS FROM EXPERIMENT 3

(Small variance values included in "Error")

Source	Degrees of freedom	Variance
Proteins (flavours, and linseed oilmeal control)	4	16.28**
APF	1	61.40**
Ustilagic acid	1	3.80
Thiouracil	1	391.70**
Proteins × APF	4	1.51
Proteins × ustilagic acid	4	2.98*
Proteins × thiouracil	4	4.86**
APF × ustilagic acid	1	
APF × thiouracil	1	6.04**
Error	58	1.875

* Significant at 5% level.
** Significant at 1% level.

THE EFFECT OF VARIOUS CANNING CROP ROTATIONS ON CERTAIN SOIL CONSTITUENTS OVER A FIVE-YEAR PERIOD[1]

H. Hill[2], R. F. Bishop[3] and H. B. Cannon[4]

Canada Department of Agriculture, Ottawa

[Received for publication September 5, 1952]

ABSTRACT

Changes that have occurred in certain soil constituents during the initial 5-year period in nine different rotations with canning peas, tomatoes and corn are discussed.

In a 2-year rotation of peas and tomatoes or peas and corn, where in addition to commercial fertilizer twelve tons of manure were applied every second year and two green manure crops were grown after harvesting the peas, an increase occurred in soil organic matter, exchangeable potassium and easily acid soluble plus adsorbed phosphorus. Results from another rotation where the corn stover was returned to the soil indicate this practice to be helpful in maintaining soil organic matter. The highest yields of corn, peas and tomatoes occurred where manure was used and the highest average yields were obtained in 2-year rotations with uninterrupted production of canning crops. Reduced yields of peas and corn occurred when clover was seeded with these crops.

Soil test values as well as the calculated additions and removals of phosphorus and potassium suggest that larger amounts of phosphorus and smaller amounts of potassium were used than was necessary for the particular area.

INTRODUCTION

Vegetable canning crops provide a major portion of the cash income of many farms in the Ontario counties of Northumberland, Durham and Prince Edward. In Northumberland county, canning crops are grown on light textured soils such as Percy fine sandy loam, Bondhead loam and Brighton sandy loam, while in Durham county the somewhat deeper, heavier textured Newcastle loam is more common. In Prince Edward county clay loams are predominant but such soils are frequently shallow, and precipitation is deficient and poorly distributed during the growing season. With the occurrence of relatively high temperatures during the cultivation period organic matter decomposes rapidly and its maintenance is difficult.

Although a small number of live stock are maintained on the majority of these farms, the amount of manure produced is insufficient to maintain soil organic matter and soil fertility with land under continuous cultivation. The problem resolves itself into adopting crop rotations which allow the highest cash income from vegetable canning crops and yet maintain soil fertility with the restricted amount of manure that is available. Present soil management practices are based on general rather than specific knowledge and on local economy. In many cases soil fertility has declined and soil erosion taken place. With this in mind a group of plots with different rotations of canning peas, tomatoes and corn was established in 1946 at the Smithfield Sub-station on Percy fine sandy loam with the purpose of

---

[1] Presented before the Soils Section, Agricultural Institute of Canada, Ottawa, June 26, 1952. Joint contribution from the Division of Horticulture, Experimental Farms Service, and the Division of Chemistry, Science Service. Scientific Contribution No. 796, Division of Horticulture and Scientific Contribution No. 228, Division of Chemistry.

[2] Principal Horticulturist, Division of Horticulture.

[3] Chemist, Division of Chemistry.

[4] Senior Horticulturist, Division of Horticulture.

investigating changes in soil constituents and crop yields over an extended period. Since the study has been in progress for a period of only five years, adequate assessment of the different rotations cannot yet be made but certain interesting trends are apparent.

MATERIALS AND METHODS

The nine rotations included in this study are described below:

Rotation 1. *Four-year rotation with one crop of peas and one crop of tomatoes.*
1st year: Peas—winter wheat and timothy sown in the fall.
2nd year: Red clover sown in the spring—crop of winter wheat harvested.
3rd year: One cut of clover hay, aftermath ploughed under.
4th year: Tomatoes.
This is a standard rotation for the district in which two canning crops are grown in each four-year period.

Rotation 2. *Five-year rotation with one crop of peas and two crops of tomatoes.* This is similar to Rotation 1 with a second crop of tomatoes grown in the fifth year of the rotation.

Rotation 3. *Four-year rotation with one crop of peas and one crop of corn.* This is similar to Rotation 1 but tomatoes are replaced by corn. All the corn stover is ploughed under with an application of 150 pounds of cyanamid per acre.

Rotation 4. *Five-year rotation with one crop of peas and two crops of corn.* This is similar to Rotation 3 with a second crop of corn grown in the fifth year of the rotation.

Rotation 5. *Four-year rotation with one crop each of tomatoes, peas and corn.*
1st year: Tomatoes.
2nd year: Peas—after the peas are harvested a green manure crop of millet is sown.
3rd year: Corn with red clover seeded in the corn at the last cultivation.
4th year: One cut of clover hay, aftermath ploughed under.

Rotation 6. *Four-year rotation with one crop of peas and two crops of tomatoes.*
1st year: Peas—red clover seeded with the peas.
2nd year: One cut of clover hay, aftermath ploughed under.
3rd year: Tomatoes.
4th year: Tomatoes.

Rotation 7. *Five-year rotation with one crop each of peas, corn and tomatoes.*
1st year: Corn, manure 12 tons per acre; corn stover ploughed under with 150 pounds of cyanamid per acre.
2nd year: Peas—winter wheat and timothy sown in the fall.
3rd year: Clover sown in the spring, crop of winter wheat harvested.
4th year: One cut of clover hay, aftermath ploughed under.
5th year: Tomatoes.

Rotation 8. *Two-year rotation with successive crops of tomatoes and peas.*
1st year: Tomatoes.
2nd year: Peas—after the peas are harvested millet is sown. Twelve tons of manure per acre are applied and the millet is disked under. This is followed by sowing fall rye.

Rotation 9. *Two-year rotation with successive crops of corn and peas.*
1st year: Corn—twelve tons of manure per acre are applied and all the corn stover is ploughed under with 150 pounds of cyanamid per acre.
2nd year: Peas—after the peas are harvested millet is sown. The millet is disked under and fall rye is sown.

Each plot occupies an area of 70 by 20 feet or approximately one thirty-first of an acre. The number of plots in each rotation corresponds to the length in years of the particular rotation. Thus, in a four-year rotation there are four plots, while in a two-year rotation there are only two plots. Each plot in a rotation was started with a different crop so that each crop included in a rotation is grown every year and the influence of season on yield can thus be eliminated.

Fertilizers

Fertilizer is applied to all canning crops, whenever they occur in the rotation. The pea crop is fertilized with 350 pounds of a 2–12–6 per acre; tomatoes with 750 pounds of a 2–12–6 per acre; and corn with 500 pounds of a 4–8–10 per acre. In addition, the grain crop receives 300 pounds of a 2–12–6 per acre. These rates are in line with the general fertilizer recommendations for the area.

Soil Analyses

Soil samples were obtained from each plot area before the commencement of the study and again in the spring of 1951 after a period of five years. Soil organic matter was determined as loss on ignition (2) and exchangeable magnesium and exchangeable potassium by extracting with neutral normal ammonium acetate at a pH of 7.0. The phosphorus status of the samples was determined by Bray's method for acid soluble and adsorbed phosphorus (1).

RESULTS AND DISCUSSION

Changes which have occurred in these soil constituents over the five-year period are given in Table 1 as "plus" or "minus" values.

Outstanding in interest is the soil improvement which has occurred in Rotations 8 and 9 with the production of a canning crop each year. Each of these rotations has produced five canning crops in this period compared to an average of three crops in the other rotations. Despite this fact there has been an appreciable increase in soil organic matter, an increase in exchangeable potassium and a marked increase in acid soluble and adsorbed phosphorus. While it is true that manure was applied in these two rotations, the annual average application of six tons per acre is modest and perhaps not beyond the supply available to the average grower whose annual canning crop acreage rarely exceeds 10 to 15 acres. It would be interesting to know to what extent the manure has been responsible for the soil improvement and to what extent the incorporation, biennially, of two green manure crops but no manure might fall short of soil fertility maintenance. It is planned to establish additional plots to secure this information. In the standard Rotation 1 without any application of manure, soil organic matter has decreased and there has been a further decrease when another crop of tomatoes was removed in Rotation 2. In similar Rotations 3 and 4, but with corn substituting for tomatoes, there has been no organic matter reduction in the four-year rotation and a smaller reduction than with tomatoes in the five-year rotation. In both these rotations all the corn stover was returned to the soil and under these conditions organic matter maintenance appeared to be a lesser problem with corn than with tomatoes. In Rotation 5 where three canning crops were removed in the four-year rotation, there has not been any greater reduction in organic matter than in Rotation 1, although one more cash crop has been removed. There is no apparent explanation for the lack of change in soil organic matter in Rotation 6. With one exception there has been an appreciable decrease in exchangeable magnesium. With the existence of initial relatively low levels of this element, some attention will have to be paid in the future to providing magnesium in the fertilizer treatment. Soil phosphorus has

TABLE 1.—AVERAGE CHANGES IN SOIL CONSTITUENTS OVER A FIVE-YEAR PERIOD

Rotation No.	Canning Crops Harvested	Organic Matter	Mg m.e./100g.	K m.e./100g.	P lb./ac.
		%			
1	1 crop of peas, 1 crop of tomatoes	−.18	−.11	0	+ 54
2	1 " " " 2 " " "	−.26	−.09	−.002	+ 98
3	1 " " " 1 " " corn	+.04	−.14	−.01	+ 45
4	1 " " " 2 " " corn	−.11	−.09	−.08	+ 54
5	1 " " " 1 " " tomatoes, 1 crop of corn	−.15	−.12	−.05	+ 76
6	1 " " peas, 2 " " "	0	−.14	−.03	+ 83
7	1 " each of corn, peas and tomatoes, 12 tons of manure applied in five years	+ .04	−.17	−.02	+ 82
8	2 crops of peas, 3 crops of tomatoes, 30 tons manure applied in five years	+1.05	+.02	+.24	+192
9	2 crops of peas, 3 crops of corn, 30 tons of manure applied in five years	+ .24	−.17	+.11	+156

increased in all rotations even though the amounts of fertilizers applied to the cash crops were but moderate. In contrast to phosphorus, there has been a decrease in exchangeable potassium except in the two-year rotations.

In Table 2, the residues or deficiencies of soil phosphorus and potassium are calculated by subtracting the amounts of these elements removed by crops from the amounts applied as fertilizer and manure (3). These in turn are compared with the actual plus or minus values obtained by soil analyses. With the exception of Rotation 4, the calculated values show that more phosphorus has been applied than has been removed by crops and this is confirmed by the build-up of acid soluble and adsorbed phosphorus in the soil, although there is no constant relationship between the calculated values and the analytical soil values. In contrast the calculated values show that in every rotation more potassium has been removed by crops than was applied in the form of fertilizer and manure. While the calculated deficiencies were much greater than the actual reductions of exchangeable potassium in the soil there was a reduction in six of the nine rotations. It would appear that there has been conversion of potassium

TABLE 2.—RELATION BETWEEN FERTILIZER APPLIED, AMOUNTS OF PHOSPHORUS AND POTASSIUM REMOVED BY THE CROPS AND CHANGES IN SOIL ANALYSES AFTER A FIVE-YEAR PERIOD

Rotation	Amounts applied as fertilizers in lb./ac.		Amounts removed by crops in lb./ac.		Residue or deficiency in lb./ac.		Plus or minus values by soil analysis in lb./ac.	
	P	K	P	K	P	K	P	K
1	87	85	62	305	+26	−245	+ 54	0
2	113	107	68	417	+44	−310	+ 98	−1.5
3	60	88	59	237	+ 1	−148	+ 45	− 16
4	65	112	78	243	−12	−130	+ 54	− 68
5	94	120	63	278	+36	−158	+ 76	− 35
6	121	115	58	413	+61	−298	+ 83	− 25
7	103	205	83	378	+19	−173	+ 82	− 11
8	181	379	108	684	+72	−305	+192	+183
9	126	390	116	396	+11	− 6	+156	+ 86

from a non-exchangeable to an exchangeable form. From this balance-sheet it would appear that fertilizers higher in potassium and lower in phosphorus might be used to advantage. This opinion is further strengthened when it is noted that the average amount of adsorbed and acid soluble phosphorus for the experimental area was 339 pounds per acre, while the exchangeable potassium was only 133 pounds per acre. In the case of tomatoes, Schmidt (4) has determined that with a phosphorus soil test of 200 pounds per acre 98.6 per cent maximum yield will be obtained without any application of phosphorus, but that a soil test of 300 pounds per acre of exchangeable potassium is required to ensure 97 per cent yield. He has further determined that with a soil test of only 133 pounds per acre of exchangeable potassium an application of 170 pounds per acre of K_2O is required for maximum yield. In this study only 45 pounds of K_2O has been applied to the tomato crop.

CANNING CROP YIELDS IN DIFFERENT ROTATIONS

It would certainly be premature to accept as significant any differences in yields of canning crops obtained from any particular rotation, since in the majority of cases the yields are those obtained before the rotation was completed the first time. Consideration of yield data may be of interest

TABLE 3.—YIELDS OF CANNING CROPS

Rotation	Average yearly wt. in lb. per plot	Average wt. in lb. per acre	Yield expressed as percentage of mean					
			1946	1947	1948	1949	1950	1951
			Corn					
3					85	116	63	79
4	236	7316	95	98	94	78	107	100
5	210	6510	96	71	95	71	86	89
7	259	8029	83	108	106	134	124	116
9	288	8928	126	122	117	100	119	116
			Shelled Peas					
1	55	1705		100	93	41	69	80
2	61	1891		76	113	83	79	67
3	59	1829		93	61	67	71	122
4	76	2356		105	105	113	91	121
5	68	2108		114	84	120	84	95
6	49	1519		36	74	60	106	72
7	88	2728		131	111	141	142	118
8	84	2604		124	126	128	117	110
9	89	2759		123	128	150	146	114
			Tomatoes					
		tons/ac.						
1	456	12.9	80	100	102	93	95	89
2	485	13.7	110	79	104	93	105	92
5	459	13.0	90	109	93	70	87	97
6	442	12.5	100	100	86	118	97	62
7	504	14.2	105	98	87	100	106	112
8	621	17.5	114	113	128	134	111	147

from a preliminary standpoint and may also indicate any tendency toward rise or decline of yield in a particular rotation. In Table 3 the yields of corn and tomatoes are given as an average for 1946–1951 inclusive, while the yields of peas are given as an average for 1947–1951. The yearly yields are expressed as a percentage of the mean yield of the crop on all plots for the year. The first point of interest is that the yields of either corn, peas or tomatoes were highest when manure was used, as in Rotations 7, 8, and 9. This is so even when only 12 tons of manure were applied in five years in Rotation 7. The highest average yields were produced by the two-year rotations with uninterrupted production of canning crops. From an economic standpoint this is all the more significant since they produced two more canning crops in this period than the other rotations. An examination of the yearly yields expressed as a percentage of the mean showed no tendency for these rotations to lose their yield superiority. In Rotation 6, where clover was seeded with the peas, the yield of peas appeared to be reduced considerably. Seeding red clover with corn also appeared to reduce the corn yield. No distinct trends towards decline or improvement of yield were shown by other rotations.

REFERENCES

1. Bray, Roger H. Correlation of soil tests with crop response to added fertilizers and with fertilizer requirement. Diagnostic techniques for soils and crops. The American Potash Institute, Washington, D.C. 1948.
2. Division of Chemistry, Science Service, Department of Agriculture, Ottawa. Chemical methods of soil analyses. Jan., 1949.
3. Ontario Department of Agriculture, Toronto, Ont. Soil management and fertilizer use. Bull. 463. Jan., 1949.
4. Schmidt, William A. Soil tests as a basis for fertilizer recommendations on tomatoes. Campbell Soup Company, West Chicago, Illinois. 1950.

OBSERVATIONS ON THE MINERAL METABOLISM OF PULLETS

X. THE EFFECTS OF GONADAL HORMONES ON RETENTION AND TURNOVER OF CALCIUM BY THE SKELETON[1]

J. R. Jowsey[2], W. F. Oliver[3], W. A. Maw[4] and R. H. Common[5]

Macdonald College, Que.

[Received for publication August 7, 1952]

ABSTRACT

Sexually immature pullets were treated during an eleven-day period with (*a*) no hormones or (*b*) testosterone propionate (TST) or (*c*) estradiol benzoate (ODB) or (*d*) estradiol benzoate plus testosterone propionate (ODB + TST). Food consumption and calcium intake were the same for all birds on a given day. Calcium in food less calcium in droppings (calcium retention) was recorded. On the eleventh and final balance day the dietary calcium was labelled by replacing the calcium carbonate supplement by a similar amount of calcium carbonate containing radiocalcium. The results *for this final day* showed (1) that ODB + TST highly significantly increased the radiocalcium retained in the skeleton, whereas TST and ODB did not; (2) that TST, ODB or ODB + TST increased the loss of calcium from the skeleton to a highly significant degree, thus demonstrating an increased exchange of calcium in the skeleton; and (3) that ODB + TST increased the turnover of skeletal calcium over all other treatments and this to a highly significant degree; while TST appeared to increase skeletal turnover, but only with a low degree of significance ($P = 0.10$).

TST did not increase net gain of calcium; ODB + TST increased net gain to a highly significant degree; and ODB significantly reduced net gain of calcium. The daily retention data for the previous ten days show that the latter effect is explicable because of changes with time in the effects of ODB on calcium retention.

A tentative hypothesis is offered to explain the marked interaction between ODB and TST in regard to calcium retention.

Certain effects of gonadal hormones on the average daily retention of calcium by the sexually immature pullet have been described previously (2), the term "retention" signifying calcium ingested less calcium in droppings. The pronounced effect of estrogen plus androgen, administered concurrently, on calcium retention (3) was confirmed. In addition it was shown that either estrogen or androgen, administered by themselves, evoked slight but significant increases in the average daily calcium retention. The present paper deals with results obtained in the same experiment from a final daily balance period in which the calcium carbonate supplement of the diet was replaced by calcium carbonate labelled with radiocalcium (Ca^{45}).

Increases of calcium retention may be due to increased deposition of calcium in the skeleton, or to decreased removal of calcium from the skeleton, or to the operation of both factors. It was hoped that labelling the food calcium during the final daily period might provide relevant information not obtainable by ordinary balance methods.

Govaerts, Dallemagne and Melon (6) have reported experiments in which radiocalcium was used to study the effects of gonadal hormones on the calcium metabolism of pigeons. The radiocalcium was given subcutaneously and its subsequent excretion in the droppings was followed.

[1] Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Que. Journal Series No. 316.

[2] Ralston Purina Research Fellow in Poultry Nutrition.
[3] Associate Professor of Physics.
[4] Professor of Poultry Husbandry.
[5] Professor of Agricultural Chemistry.

Govaerts *et al.* (6) were able to demonstrate that estrogen increased the proportion of the injected calcium that was retained by the bones. Estrogen also increased the physiological half-life of the radiocalcium from about 45 days in the control birds (unestrogenized males) to about 75 days in the estrogenized birds (males). It was shown that the femur and tibia of estrogenized birds had a greater relative capacity for retaining radiocalcium than breast bone, radius or ulna. The experimental work at present described has covered somewhat similar ground, but the radiocalcium has been given in the food and the work has included determination of calcium balances and analyses of the entire skeletons.

MATERIAL AND METHODS

General

The birds were sexually immature pullets (New Hampshire males × Barred Plymouth Rock females). The procedure for feeding, feeding schedule and the balance technique have been described previously (2). Uniformity of daily calcium and food intake and regularity of feeding schedule are specially important in experiments of this kind

Four groups each of three sexually immature pullets received each day a similar quantity of mash and calcium carbonate during a 5-day preliminary balance period and a subsequent 11-day experimental period. The experiment was carried out twice, the entire data being considered together.

The experimental treatments were as follows:

Group 1. No hormones.

Group 2. 0.75 mgm. testosterone propionate (TST) on alternate days.

Group 3. 1.00 mgm. estradiol benzoate (ODB) on alternate days

Group 4. 1.00 mgm. ODB plus 0.75 mgm. TST on alternate days.

The hormones were dissolved in similar volumes (0.5 ml.) of sesame oil and were administered by intramuscular injection. Group 1 was given a similar amount of oil only.

On the day of the sixth and last injection the birds were fed as usual, except that the calcium of the food was labelled by replacing the calcium carbonate allowance (2.50 gm.) by an equal weight of calcium carbonate containing Ca^{45}.

On the following morning, and at the usual hour of feeding, the birds were lightly anaesthetized by intravenous nembutal and rapidly bled by cutting the veins in the roof of the mouth. The bodies were skinned, eviscerated, and the carcasses quick-frozen pending analysis. The contents of the digestive tract and the droppings for the previous 24 hours were reserved separately for determinations of calcium and radioactivity. The skeletons were freed from muscle and connective tissue, and also analysed for total calcium and radioactivity. Muscle connective tissue, and viscera, other than the alimentary tract, were discarded. Determinations of radioactivity on samples of these tissues, together with previous observations, showed that the radiocalcium content of these materials was negligible in relation to measurements of balance and of total radiocalcium in the skeletons.

Determination of Radiocalcium

The radioactive calcium carbonate was prepared by bombardment of "Specpure" (Johnson Matthey and Mallory, London) calcium carbonate in the nuclear reactor of the National Research Council of Canada. This material had an activity ("specific activity") of 2058 counts per min. per mgm. Ca.

Samples for determination of radioactivity were precipitated as the oxalate and transferred to copper planchets by a filtration technique described by Fiskell (4). Radioactivity was determined using a thin mica-window Geiger-Mueller tube. All counts were corrected for background, self-absorption and also for changes in counter efficiency. The resolving time of the counter was checked by the method described by Henneberry (8).

Analytical Methods

Samples free from radiocalcium were prepared for analysis by ashing in the muffle furnace and dissolving in hydrochloric acid. Samples containing radiocalcium were ashed by prolonged boiling with concentrated nitric acid, in order to avoid any risk of contaminating the muffle with radioactive calcium. Gentle heating of bone samples with nitric acid was continued until the organic matrix was largely decomposed. The bulk of the nitric acid was then evaporated, the residue diluted to a suitable volume with water and aliquots taken for completion of ashing by means of perchloric acid.

Recoveries of Radioactivity

The pertinent data for the birds in the present experiment are set out in Table 1. The data reveal a satisfactory recovery.

TABLE 1.—PERCENTAGE RECOVERY OF RADIOACTIVITY FED TO THE EXPERIMENTAL PULLETS

Group	Number of pullets	Radioactivity of skeletons, droppings and gut contents expressed as a percentage of the radioactivity in the food eaten	
		Total recovery per cent	Range of recoveries for individual pullets per cent
1	6	100.6	99–102
2	5*	98.2	96–100
3	6	99.0	94–103
4	5*	99.8	96–101

* The droppings of one bird in Group 2 and of one bird in Group 4 were lost during ashing, so that recoveries could not be determined.

Experimental Results and Discussion of Results

The experimental data included the calcium and radioactivity contents of the food, of the droppings and of the contents of the gut. The dietary intake of calcium comprised 992 mgm. Ca from the radioactive calcium carbonate and 127 mgm. Ca from the other dietary ingredients. All this food calcium would come into solution rapidly during digestion. The total dietary calcium could, therefore, be regarded as labelled, and as carrying an activity of 1825 counts/min./mgm. Ca (i.e. 2058 ÷ 1.119), representing a total activity of 2042 $\times$ 10^3 counts/min.

The amounts of the calcium of gut and of droppings which had originated from that day's intake of calcium could be computed from these data (*vide* Table 2). The total Ca of droppings and gut, however, included also calcium derived from somewhere else than that day's intake. Those amounts of calcium other than food calcium are shown in Table 2, lines 5 and 6. There were two possible sources of this calcium:—(*a*) calcium present in the gut at the time the last daily food allowance was given; and (*b*) the body calcium, which is practically synonymous with skeletal calcium for the purposes of balance studies.

It was not possible directly to determine the portion of the "Ca other than food Ca" in droppings and gut which ought to be ascribed to calcium in the gut at the time of feeding. However, the pullets were in the post-absorptive state at the time of feeding and a strict time-schedule was observed. It can be assumed for purposes of calculation that the calcium content of the gut at the time of feeding was, on the average, the same as at the time of slaughter. The calcium of the gut at the time of slaughter had a relatively low specific activity. This suggests that it was largely excretory. Calcium absorbed from the day's intake would be labelled; and such

TABLE 2.—EFFECTS OF GONADAL HORMONES ON CALCIUM METABOLISM OF THE SEXUALLY IMMATURE PULLET. AVERAGE DATA FOR ELEVENTH AND FINAL BALANCE DAY

	Treatment	Group 1 Control	2 TST 6 × 0.75 mgm.	3 ODB 6 × 1.0 mgm.	4 ODB 6 × 1.0 mgm. + TST 6 × 0.75 mgm.	F.	LSD P = 0.05	LSD P = 0.01
		Number of pullets 6	5*	5**	5*			
		mgm.	mgm.	mgm.	mgm.			
1	Ca in gut, post absorptive	60	117	88	65	—	—	—
2	Ca in food, labelled	1,119	1,119	1,119	1,119	—	—	—
3	Food Ca from gut	9	14	12	18	—	—	—
4	Food Ca from droppings	915	874	931	739	—	—	—
5	Ca other than food Ca from gut (1) − (3)	50	102	76	48	—	—	—
6	Ca other than food Ca from droppings	29	77	88	102	—	—	—
7	Food Ca gained by skeleton (2) − (3) − (4)	195	231	176	352	12.145	73	100
8	Presumptive loss of Ca from skeleton (5) + (6) − (1)	19	62	76	85	9.730	39	53
9	Net gain of Ca by skeleton (7) − (8)	176	168	100	274	12.929	60	83
10	Turnover of skeletal Ca (7) + (8)	214	293	252	447	7.893	108	148
11	Final total skeletal Ca	12,310	—	12,270	14,680	—	—	—

* Results not available for one bird owing to loss of sample.
** Results for one bird omitted because of extreme deviations (more than 3 × SE) from group mean for day's retention and other data.

of this calcium as was absorbed and entered the bones by exchange would, in the first instance, have displaced non-labelled Ca. Even at the end of the absorptive period, the calcium leaving the bones would be of very low activity compared with the food calcium because of the dilution effect in the skeleton. The "Ca other than food Ca" of the gut can, therefore, be regarded as displaced from the bones by exchange with absorbed food Ca. The rest of the calcium displaced from the bones must have been in the droppings, together with most of the "Ca other than food Ca" derived from calcium present in the post-absorptive gut at the time of feeding.

It is reasonable, therefore, to regard the "Ca other than food Ca" in droppings and gut, less an amount of calcium equal to that assumed to be present in the gut at the time of feeding, as providing a measure of calcium displaced from the skeleton by exchange. There is not any other place from which this calcium could have come. This quantity may be termed the "presumptive loss" (i.e. the gross loss) of calcium from the skeleton. The relevant average data are presented in Table 2, line 8. The difference between the food Ca gained by the skeleton (i.e. the gross gain) and the presumptive loss of Ca from the skeleton then gives a measure of the net gain of Ca by the skeleton, while their summation gives a measure of the turnover (i.e. gross gain plus gross loss) of skeletal calcium (Table 2, lines 9 and 10).

The "F" values obtained by analysis of variance showed that highly significant differences existed between groups in respect of food Ca gained by skeleton, presumptive loss of Ca from skeleton, net gain of Ca by skeleton and turnover of skeletal Ca.

Food Ca gained by the skeleton was highly significantly increased by estrogen plus androgen, but not by estrogen alone, or by androgen alone. This might have been expected from previous findings (3, 2).

The effects of estrogen alone and androgen alone on average daily retention are slight compared with the effect of estrogen plus androgen (2) and might, therefore, be obscured by other sources of variance in a series of observations restricted to one balance day.

The "presumptive loss of Ca" from the skeletons of the control birds was lower than for the other groups to a highly significant degree. The other three groups did not differ significantly from one another in this respect. The important and unexpected feature of these results is the increase of "presumptive loss of Ca" associated with treatment with androgen alone. This suggests strongly that the androgen treatment was associated with increased turnover of skeletal Ca. The present results do, in fact, suggest that the turnover in Group 2 was greater than in Group 1, but the difference was of a low degree of significance (just short of significance at $P = 0.10$). Turnover was increased to a highly significant degree by estrogen plus androgen, the interaction between estrogen and androgen in this respect being highly significant. The data for net gains of Ca by the skeleton reveal a highly significant positive effect of estrogen plus androgen. The data also reveal a significant negative effect of estrogen; this feature of the experimental results might appear anomalous at first sight, in view of the fact that androgen and estrogen both slightly increased the average daily retention of calcium over the ten-day experimental

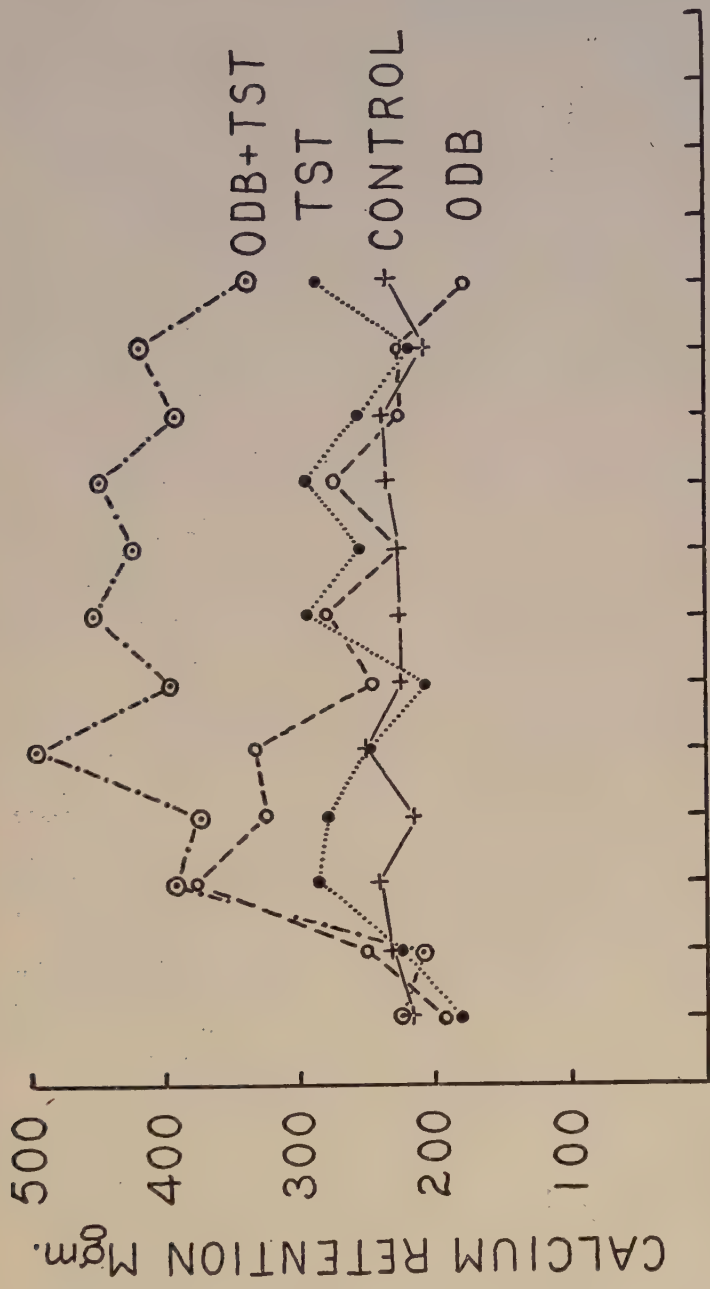


Figure 1. Effect of hormones on Ca retention by the immature pullet. Injections on days 1, 3, 5, 7, 9, 11.

period (2). The anomaly disappears when the time sequence of treatment effects is taken into consideration. Figure 1 presents the data for average calcium *retention* by the different groups over the treatment period. Retention by the control group remained relatively constant throughout; retention by the birds treated with androgen was more variable from day to day and remained slightly higher than in the control birds. The birds receiving estrogen displayed a significant increase in calcium retention during the second and third days; on the fourth day an evident tendency of retention to decline again led to a lowered significance of the increase (only significant at $P = 0.10$). Thereafter the decline continued, and the significant decrease associated with estrogen on the eleventh day is thus consistent with the previous trend of the balances for this group. Retention by the birds receiving estrogen plus androgen was increased to a significant degree and remained significantly higher than retention by the other groups throughout. Even here, however, a tendency to a decline in the later phases is evident. It is possible that the results with estrogen, and to a less degree also the results with estrogen plus androgen, are a reflection of the known tendency of birds to become less responsive to estrogen with continued administration (9). It would seem likely, therefore, that adminstration of the labelled calcium carbonate after a 4-day treatment period instead of after a 10-day treatment period, might have revealed a significant *positive* effect of estrogen on net gain of Ca by the skeleton instead of the significant negative effect observed in the experiment as reported. The observations serve to emphasize the advisability of studying the day-to-day course of calcium exchanges in balance studies of this kind.

Tentative Conclusions

Any interpretation of the results obtained using radiocalcium should take cognizance of the following facts:—

(*a*) that estrogen, and also estrogen plus androgen, greatly increase serum calcium while androgen does not;

(*b*) that estrogen, and also estrogen plus androgen, stimulate the formation of endosteal bone, the action possibly being local (1), but that androgen does not;

(*c*) that estrogen and androgen both tend slightly to increase the average daily retention of calcium by the pullet in experiments extending over a 10- to 12-day treatment period, but that this effect is small compared with the large increase in retention evoked by estrogen plus androgen; and

(*d*) that, as discussed above, estrogen alone appears to increase calcium retention to an appreciable degree during the early phases of estrogen treatment, but that this positive effect may disappear with time and may even become a negative effect.

A tentative hypothesis may be stated as follows: Estrogen in the first instance stimulates formation of endosteal osteoid tissue (1) and thus provides a new locus for calcium deposition. At the same time the estrogen stimulates the liver to produce constituents which are passed to the blood

(5). These constituents enhance the calcium-binding capacity of the blood and lead to increased serum calcium and, thereby, enhance the capacity of the blood for calcium transport. These effects lead to an initial increase of calcium retention, but the effects decline as the bird tends to become refractory to estrogen (9).

Androgen increases calcium retention slightly, although it does not increase serum calcium or stimulate new endosteal bone formation. However, since androgen increases loss of calcium from the bones (*vide supra*), and probably increases turnover of skeletal calcium, it seems possible that androgen may stimulate increased absorption of calcium from the gut, or else increase the metabolic activity of the bone cells. In the absence of evidence to the contrary, the former possibility may seem the simpler explanation; the fact that there is some indirect evidence that androgen may increase the absorption of carotenoids by the fowl lends some support to this view (7). If this is indeed the case, then the marked interaction of androgen and estrogen in respect of increasing retention of calcium from the food is explicable as due to interaction of an increased capacity for absorption induced by androgen with an increased formation of new endosteal bone induced by estrogen.

The foregoing hypothesis is tentative and provisional, but it would seem to accord with the known facts regarding the effects of gonadal hormones on calcium metabolism.

A Note on Direct and Indirect Estimates of Food Calcium Retained by the Skeleton

In the present experiment it was possible to test the validity of the indirect estimates of food Ca in the skeletons calculated from the balance data (Table 2, line 7), because the skeletons of the birds were also analysed for radioactivity at the conclusion of the experiment. The correlation coefficient between the indirect and direct estimates was = 0.950 for 20 d.f. The regression equation was

$$Y = 1.025\ X - 0.46$$

where Y = indirect estimate of labelled calcium in skeleton

and X = direct estimate of labelled calcium in skeleton.

In the present paper the indirect estimates have been used. The foregoing correlation affords a basis for considerable confidence in the accuracy of the indirect estimates.

ACKNOWLEDGMENTS

The authors wish to thank the Ralston Purina Company for the award of a Research Fellowship to J. R. Jowsey, which made it possible to attack the problems concerned on a more comprehensive basis than would otherwise have been possible. They also wish to thank Messrs. Schering, Limited, Montreal, for generous gifts of hormones. The financial support of the National Research Council of Canada is gratefully acknowledged.

The authors are further indebted to Mrs. J. R. Jowsey for technical assistance throughout, to Howard Steppler, Macdonald College, for guidance on statistical questions, and to B. B. Migicovsky and A. R. G. Emslie, Canada Department of Agriculture, for invaluable criticism and suggestions.

REFERENCES

1. Benoit, J., and J. Clavert. Action ostéogénétique directe et locale de la folliculine, démontrée, chez le canard et le pigeon, par son introduction localisée dans un os long. C. R. Soc. Biol. 139 : 728-730. 1945.
2. Common, R. H., J. R. Jowsey, and W. A. Maw. Observations on the mineral metabolism of pullets. IX. Effect of estrogen and androgen administered separately on the retention of calcium by the sexually immature pullet. Can. J. of Agr. Sci. 33 : 172-177. 1953.
3. Common, R. H., W. A. Rutledge, and R. W. Hale. Observations on the mineral metabolism of pullets. VIII. The influence of gonadal hormones on retention of calcium and phosphorus. J. Agr. Sci. 38 : 64-80. 1948.
4. Fiskell, J. G. A. The use of radioactive phosphorus in the investigation of fixation and release phenomena in soils. M.Sc. thesis, McGill University. 1949.
5. Fleischmann, W., and I. A. Fried. Studies on the mechanism of the hypercholesterolemia and hypercalcemia induced by estrogen in immature chicks. Endocrinology 44 : 283-286. 1946.
6. Govaerts, J., M. J. Dallemagne, and J. Melon. Radiocalcium as an indicator in the study of the effect of estradiol on calcium metabolism. Endocrinology 48 : 443-452. 1951.
7. Gardner, V. E., W. E. Phillips, W. A. Maw, and R. H. Common. Vitamin A ester and vitamin A alcohol in the serum of the oestrogenized immature pullet. Nature 170 : 80. 1952.
8. Henneberry, G. O. Synthesis of phenylalanine labelled with C^{14}. M.Sc. thesis, McGill University. 1950.
9. Mandel, P., J. Clavert, and Mme. P. Mandel. Effets de la folliculine sur le phosphore plasmatique acido-soluble chez le canard. C. R. de la Soc. Biol. 141 : 515-517. 1947.

A STUDY OF SOIL AND FERTILIZER PHOSPHORUS UPTAKE ON A CALCAREOUS SOIL BY OAT PLANTS, USING RADIOACTIVE TRACER TECHNIQUE[1]

D. D. Dolson[2] and J. W. Ketcheson[3]

Ontario Agricultural College, Guelph, Ont.

[Received for publication July 17, 1952]

ABSTRACT

Two series of greenhouse experiments were carried out to establish the over-all response and the response to starter applications of phosphate fertilizer applied to Bondhead loam and Bondhead silt loam, as indicated by oat plants. Monocalcium phosphate labelled with P^{32} was used as a source of phosphorus. Dry weight, total phosphorus absorption, soil phosphorus and fertilizer phosphorus absorption data were collected to determine the responses obtained.

The results of these experiments show that a positive response was obtained from the starter treatments. The over-all response to complete treatment was established, and was shown to be greater than that from the starter treatment. When phosphorus was applied to the soil at the rate of 80 p.p.m. P, the first significant increases found four weeks after germination resulted from treatment of a layer of soil one inch in depth. More extensive treatment gave significantly greater increases. The effects of fertilization on soil phosphorus utilization varied with treatment and stage of development.

Many of the soils of Southern Ontario are known from practical experience to be relatively low in readily available phosphorus, and to show a greater or lesser degree of response to added phosphatic fertilizers. When applying phosphatic supplements to the soil, the common practice in Ontario has been to place the fertilizer, at planting time, in a limited area in close association with the seed. Thus a "starter effect" is obtained. The roots of the young plants have access immediately to a zone of soil relatively rich in phosphorus. As growth proceeds, the root feeding zone is extended and the plant roots are no longer entirely within the phosphorus-enriched soil. An alternate method of application is to apply sufficient supplement to raise the entire root feeding zone to a fertility level comparable to that produced in the early stages of growth by a starter application. This latter method of application should not be required if the soil can supply the plant's phosphorus requirement after the starter effect has diminished, and if the starter application supplies sufficient phosphate to maintain the existing level in the soil.

This present paper is a report on a greenhouse investigation of the effects of starter and complete applications of phosphate fertilizer applied to a high-lime soil.

MATERIALS AND METHODS

The soils used in this investigation were two samples of surface soil (Ac horizon) collected in York County from areas mapped in the Bondhead series by the Ontario Soil Survey. The Bondhead soils have developed from medium-textured till materials of dominantly Trenton Limestone origin.

[1] Contribution from the Department of Soils, Ontario Agricultural College, Guelph, Ontario. Part of a thesis submitted to the School of Graduate Studies, University of Toronto.

[2] Graduate Student, Department of Soils, O.A.C.; at present with Canadian Industries Limited, Chatham, Ontario.

[3] Assistant Professor, Department of Soils, O.A.C.

One sample was a silt-loam in texture, very high in water-soluble calcium, medium in exchangeable potassium, low in water-soluble magnesium, and very low in 0.05 N acid-soluble phosphorus. The other sample was a loam in texture (49 per cent sand), high in calcium, medium in potassium and magnesium, and medium to low in phosphorus.

A more detailed description of the Bondhead soils and their agricultural use has been published by Webber *et al.* (1).

Phosphorus fertilizer was applied to the soil in the form of monocalcium phosphate monohydrate labelled with P^{32} as a tracer. The labelled fertilizer was prepared by precipitation from a solution, to which had been added a certain amount of the pure radioactive isotope. The precipitation technique described by Larson (2) was used. In all cases potash was supplemented to ensure adequacy and adequate nitrogen levels were maintained throughout the growth period by additions of NH_4NO_3 in the water as required.

Experiment I

Monocalcium phosphate monohydrate labelled with P^{32} was added to the soil in two-gallon glazed pots in two ways:

(1) mixed at the rate of 80 p.p.m. P with the top one inch of soil (starter treatment) and,

(2) mixed at the same concentration with all the soil in the pot (complete treatment).

Check pots were employed and all treatments were laid out in triplicate. Mixing was accomplished by adding the fertilizer material to $\frac{1}{4}''$ screened and air-dry soil, and rotating in a metal drum fitted with baffles.

Fifteen uniform plants of Clinton oats were grown per pot. The plants were harvested at two stages of growth for the purpose of determining the responses obtained, (1) four weeks after germination, (2) twelve weeks after germination, just prior to maturity. All harvests were carried out by removing the above-ground portion of all plants in each pot.

Experiment II

A second series of experiments was laid out in order to better characterize the starter effect of phosphate fertilizers. Four replications of six treatments on each soil were prepared in flats 4 inches in depth. Labelled monocalcium phosphate monohydrate was mixed at the rate of 80 p.p.m. P with layers of soil of varying depths: (1) four inches, (2) two inches, (3) one inch, (4) one-half inch, (5) one-quarter inch. Checks were employed on each soil. In all cases, the soil in which the seed was to be planted received the treatment.

Forty-five uniform plants of Clinton oats were grown per flat. The above-ground portion of all plants on each flat was harvested four weeks after germination.

Plant Analyses

The oven-dry samples were weighed for dry weight determination. The dried material was ashed and total phosphorus determined on an aliquot of the ash solution by the phosphomolybdenum blue reaction using Elon as the reducer. Intensity of colour development was measured with an Evelyn photo-electric colorimeter fitted with 660 mμ filter.

The amounts of fertilizer phosphorus taken up were determined by measuring the radioactivity of the ash solution in comparison to a standard of the original fertilizer material containing a known amount of phosphorus. Radiation measurements were made with a Geiger-Mueller solution-counting tube and automatic scaler and timer. Standard counter geometry was maintained throughout.

Native soil phosphorus taken up by the plants was determined by difference.

RESULTS AND DISCUSSION

Experiment I

The results of this experiment show a positive response to phosphate applications on each soil.

At the 4-week harvest on the silt loam soil, the starter treatment gave a highly significant increase over the check in total dry weight and in total phosphorus uptake. Similar increases were obtained on the loam soil. However, complete treatment gave further highly significant increases in total phosphorus uptake on each soil. The total dry weight of plant material on completely treated pots was slightly higher than on those receiving starter treatment, although no statistically significant difference at the 5 per cent point was detected on the Bondhead loam.

TABLE 1.—TOTAL DRY WEIGHT AND TOTAL PHOSPHORUS UPTAKE BY OATS AS RELATED TO TREATMENT ON TWO SOILS AT TWO STAGES OF GROWTH

Method of phosphate application	Bondhead silt loam				Bondhead loam			
	4 weeks		12 weeks		4 weeks		12 weeks	
	Dry weight per pot	Total P uptake per pot	Dry weight per pot	Total P uptake per pot	Dry weight per pot	Total P uptake per pot	Dry weight per pot	Total P uptake per pot
	gm.	mgm.	gm.	mgm.	gm.	mgm.	gm.	mgm.
Check	0.93	1.16	3.86	7.48	1.42	2.51	6.22	13.68
Starter	1.37	2.83	6.13	10.97	1.69	3.90	7.16	14.63
Complete	1.52	6.67	7.81	19.06	1.76	7.52	8.25	21.74
L.S.D. (P = 0.05)	0.13	0.72	0.71	2.54	0.10	0.61	0.68	2.75
L.S.D. (P = 0.01)	0.21	1.19	1.18	4.22	0.16	1.02	1.13	4.56

At the 12-week stage of growth the pots of silt loam soil receiving starter treatment gave higher total dry weight and greater phosphorus uptake than did the checks, although the per cent phosphorus in the dry material decreased slightly. The complete treatment, however, gave a much greater total dry weight and total phosphorus uptake than did the other treatments. On the Bondhead loam, the starter treatment increased the yield of dry matter but gave no significant increase in total phosphorus uptake. The complete treatment, on the other hand, resulted in a large increase in phosphorus uptake over the starter and a significant increase in dry weight.

These results would indicate that a starter phosphate application to a depth of one inch in these soils does produce a definite positive response in oats as measured by total dry weight of plant material produced and total phosphorus uptake. However, complete treatment of these soils with the same concentration of added phosphorus produces a greater response.

Experiment II

In Experiment I, the gross response of these soils to added phosphate was established and a starter response was demonstrated. Experiment II was designed to determine the depth of layer required to be fertilized in order to obtain the starter response.

The results of this experiment are summarized in Table 2.

TABLE 2.—TOTAL DRY WEIGHT AND TOTAL PHOSPHORUS IN THE DRY MATTER OF OATS AS RELATED TO DEPTH OF FERTILIZATION ON TWO SOILS

Depth of fertilized layer	Bondhead silt loam		Bondhead loam	
	Dry weight per flat	Total P uptake per flat	Dry weight per flat	Total P uptake per flat
inches	gm.	mgm.	gm.	mgm.
4	1.97	11.58	3.33	17.10
2	1.82	6.23	2.85	8.77
1	1.72	4.30	2.56	6.03
½	1.65	3.23	2.45	4.89
¼	1.64	3.02	2.47	5.07
Check	1.75	3.27	2.30	4.69
L.S.D. (P = 0.05)	0.21	1.16	0.15	0.62
L.S.D. (P = 0.01)	0.29	1.60	0.21	0.86

On the Bondhead silt loam only the full depth treatment gave a significant dry weight increase over the check. However, progressive increases in total phosphorus uptake occurred when layers of one-inch depth and greater were fertilized. On the Bondhead loam, all treatments gave increases in dry weight over the check. No significant difference was noted between the one-quarter, one-half, and one-inch layers but two- and four-inch layers gave successive significant increases. Again the one-inch layer was the shallowest which increased total phosphorus uptake. Further successive increases in uptake occurred as the depth was increased.

From these results, it would appear that the effects of a starter application of phosphate fertilizer applied at a concentration of 80 p.p.m. phosphorus in these soils are not manifest in oats grown in the greenhouse until the depth of treatment reaches one inch. However, further responses are obtained during a four-week period of growth when the fertilized layers are two and four inches in depth.

EFFECT OF TREATMENT ON SOIL PHOSPHORUS UTILIZATION

Since the phosphate fertilizer used in these experiments was labelled with P^{32} it was possible to determine what proportion of the phosphorus in the plant was absorbed from native soil forms and what proportion from the added fertilizer.

In Experiment I (Table 3) the uptake of soil phosphorus was increased by the starter treatment and further increased by complete treatment at the 4-week harvest. At the later harvest, however, each treatment caused a decrease in soil phosphorus uptake, the most severe decrease resulting from complete treatment. At each stage of growth, the amount of fertilizer phosphorus taken up increased with increases in the amount of fertilizer applied.

In Experiment II (Table 4), as the depth of the fertilized layer increased the amount of fertilizer phosphorus taken up also tended to increase. The increase was small up to and including the half-inch depth. Successive increases were obtained as the depth of the fertilized layer was further increased. Soil phosphorus uptake also varied with treatment. Little change occurred as the depth of the fertilized layer increased from zero to one inch. The 2- and 4-inch layers of fertilization resulted in successive decreases in soil phosphorus uptake.

TABLE 3.—SOIL AND FERTILIZER PHOSPHORUS UPTAKE BY OATS AS RELATED TO TREATMENT ON TWO SOILS AT TWO STAGES OF GROWTH

Method of phosphate application	Bondhead silt loam				Bondhead loam			
	4 weeks		12 weeks		4 weeks		12 weeks	
	Fert. P	Soil P	Fert. P	Soil P	Fert. P	Soil P	Fert. P	Soil P
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Check	—	1.16	—	7.48	—	2.51	—	13.68
Starter	1.11	1.72	5.43	5.54	0.84	3.06	3.16	11.47
Complete	3.76	2.91	15.51	3.55	3.23	4.29	15.05	6.69

TABLE 4.—SOIL AND FERTILIZER PHOSPHORUS UPTAKE BY OATS AS RELATED TO DEPTH OF FERTILIZATION ON TWO SOILS

Depth of fertilized layer	Bondhead silt loam		Bondhead loam	
	Fertilizer P uptake	Soil P uptake	Fertilizer P uptake	Soil P uptake
inches	mgm.	mgm.	mgm.	mgm.
4	9.43	2.15	13.92	3.18
2	4.26	1.97	5.10	3.67
1	1.89	2.41	1.11	4.92
½	0.46	2.77	0.25	4.64
¼	0.40	2.62	0.12	4.95
Check	—	3.27	—	4.69

SUMMARY

Two greenhouse experiments were conducted, using oat plants as the indicator crop, to determine the effects of starter and complete applications of phosphate fertilizer to a high-lime soil.

In Experiment I, the addition of starter applications of monocalcium phosphate gave significant increases in total dry weight and total phosphorus uptake at 4 weeks and 12 weeks after germination. Further significant increases were obtained when the entire root feeding zone was fertilized.

In Experiment II, starter applications of monocalcium phosphate applied to soil layers less than one inch in depth gave no significant increase in yield or total phosphorus uptake 4 weeks after germination. Treatment of a one-inch-deep layer produced significant increases in dry weight and total phosphorus uptake. More extensive treatment gave further increases.

The effect of fertilization on soil phosphorus utilization varied with treatment and stage of development.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to R. S. Brown, Professor and Head of the Department of Chemistry, Ontario Agricultural College, for the very willing co-operation received and for the use of the radioactive laboratory and equipment.

Appreciation is expressed, likewise, to Canadian Industries Limited, and to the Consolidated Mining and Smelting Company of Canada, Limited, whose financial assistance helped make this investigation possible.

REFERENCES

1. Webber, L. R., F. F. Morwick, and N. R. Richards. Soil survey of Durham County. Report No. 9 of the Ontario Soil Survey. Experimental Farms Service, Canada Department of Agriculture, Ottawa, and the Ontario Agricultural College, Guelph. 1946.
2. Larson, H. W. E. Preparation and properties of mono-, di-, and tricalcium phosphates. Ind. and Eng. Chem., *Anal Ed.*, 7 : 401–406. 1935.

CONSUMPTIVE USE OF WATER IN ORCHARD SOILS[1]

II. EFFECTS OF EVAPORATING POWER OF AIR AND OF LENGTH OF IRRIGATION INTERVAL[1]

J. C. Wilcox, J. L. Mason and J. M. McDougald[2]

Experimental Station, Summerland, B.C.

[Received for publication July 16, 1952]

ABSTRACT

The rate of consumptive use of water was determined to a depth of six feet in the soil in 41 plots of mature McIntosh apple trees in five districts. Three locations were sampled in each plot, each being half-way between the trunk of a tree and the centre of the tree square. Other consumptive use tests were made every few feet across the diagonals of tree squares. Records were also obtained, in each district, of the rate of evaporation from an open water tank 12 inches in diameter and 6 inches deep. The major findings were as follows:

(1) Considerable variation in rate of consumptive use was found to occur from point to point across the diagonal of a tree square; (2) Great variability in rate of consumptive use was encountered among the three locations used in each tree square; (3) A highly significant positive correlation was obtained between the rate of evaporation from an open water tank and the rate of consumptive use; (4) A significant negative correlation was obtained between the length of irrigation interval and the average daily rate of consumptive use; (5) The curve obtained by plotting the logarithm of the length of irrigation interval against the maximum rate of evaporation from an open tank during the season proved to be a straight line with a negative coefficient. The shorter the interval, the greater was the daily average of its greatest seasonal evaporation; (6) The maximum consumptive use during any one length of irrigation interval ranged from 0.435 inch per day with a 5-day interval to 0.153 inch per day with a 40-day interval. For some unknown reason, these values are as high as those obtained for actual irrigation requirements.

The first paper of this series (15) dealt with the effects of depth in the soil on the rate of consumptive use of water from orchard soils. This present paper deals chiefly with the effects of (1) the evaporating power of the air, and (2) the length of the irrigation interval, on the rate of consumptive use. A brief report is also made of the variability in consumptive use across a panel from one row of trees to the next.

REVIEW OF LITERATURE

A number of workers have investigated the effects of distance from a fruit tree on the rate of consumptive use of water from the soil, and on the relationship of this to the concentration of fibrous roots in the soil. Aldrich, Work and Lewis (2), working with pear trees in Oregon, found considerable variations in rate of consumptive use with greater distance from the tree. On the average, the area of greatest consumptive use was at a distance of 4 to 8 feet from the trunk. A close correlation was found between the rate of consumptive use and the concentration of fibrous roots. Veihmeyer and Hendrickson (12), working with walnut, peach and prune trees in California, found very little effect of distance from the tree on moisture use from the soil. Schuster and Stephenson (9), working with walnut trees in Oregon,

[1] Contribution No. 794 from the Division of Horticulture, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Canada. The major part of this paper was presented at meetings of the Northwest Section of the American Society of Agricultural Engineers, held in Moscow, Idaho, October 25–27, 1951.

[2] Senior Horticulturist, Horticulturist, and Assistant Technician, respectively, in charge of plant nutrition, soils and irrigation investigations.

found the highest concentration of fine roots to be within five feet of the trunk. They attributed this primarily to lack of deep cultivation near the trunk. Aside from this, the concentration of fine roots appeared to be affected more by soil conditions than by distance from the trunk.

Numerous investigators have studied the relationships between meteorological factors and the rate of transpiration from plant leaves. Temperature, humidity, wind and sunshine have all been shown (5, 6) to influence the rate of transpiration. Smith, Kinnison and Carns (11) in Arizona obtained a good correlation between the rate of evaporation from an open water tank and the rate of consumptive use by citrus trees early and late in the season, but not during the heat of the summer. Aldrich and Work (1) in Oregon reported a positive relationship between the rate of evaporation and the rate of transpiration, and a negative relationship between the rate of evaporation and the rate of enlargement of pear fruits. Little work appears to have been done, however, on the relationship between the rate of evaporation from an open water tank and the rate of transpiration from deciduous fruit trees.

There appears to be little reference in the literature to the effects of the length of the irrigation interval on the rate of consumptive use of water from the soil. Occasional reference is found to the fact that during any stated period following an irrigation, the rate of evaporation from the surface soil is usually greater from a heavy soil than from a sandy soil (10); and that with any one type of soil, more total water is lost by evaporation during the season when the irrigation interval is short than when it is long (8). No reference was found in which these factors were applied to rate of consumptive use of water from the soil. Occasional reference is also made (3, 8, 14) to the fact that sandy soils require more actual irrigation water during the season than do heavy soils, because of the greater wastage of water involved in irrigation. Israelsen (3) in Utah found a good negative correlation between the field capacity of the soil for water and the amount of irrigation water used during the season by growers. Pair and Criddle in Idaho (7) have recently established a curve for use in adjusting water requirements upwards as the amount of water required per irrigation decreases.

PROCEDURE

Consumptive use tests were made in mature apple orchards in 1950, 41 tests being made in 32 plots. The procedures used in the selection of sampling locations and in the sampling of the soil were described in the first paper of this series (15). Each plot consisted of four trees in a square pattern. Three sampling locations were selected in each plot, each location being half-way between the trunk of a tree and the centre of the tree square. Soil samples were taken following an irrigation and again prior to the next irrigation; and from these the rate of consumptive use of water was determined. It was expressed in terms of inches of water per day. No plots were used where the soil contained gravel, or where seepage water appeared prior to or during the irrigation season.

When the soil samples were taken, no compositing was done. It was thus possible to determine the rate of consumptive use for each depth at each location. For purposes of correlation with factors affecting con-

FIGURE 1. Open tank evaporimeter, 12 inches in diameter and 6 inches deep, such as was used for determining evaporating power of the air.

sumptive use, however, the total consumptive use to a depth of six feet was determined for each of the three locations in each plot, and these totals were averaged to represent the plot as a whole.

It was anticipated that the consumptive use values obtained by this procedure would be correlated with various factors. It was considered desirable, therefore, to determine how closely the sampling position used represented the average of the tree square as a whole. In an attempt to answer this, consumptive use tests were made every five or six feet from one tree to another across the diagonal of a tree square. This was done in seven orchards.

In order to obtain records on rate of evaporation from an open water surface, evaporimeter tanks were set out in each of the following districts early in the summer: Osoyoos, Summerland, Glenmore, East Kelowna, Winfield and Vernon. Each evaporimeter consisted of a galvanized iron cylindrical tank 12 inches in diameter and six inches deep (Figure 1). It was sunk in the ground to a depth of four inches, and the water surface was maintained at ground level by measured daily additions. The water was protected with one-inch chicken wire mesh. The rate of evaporation from each of these tanks was determined throughout the irrigation season, and the records obtained were used to calculate the average daily rate of evaporation applicable to each irrigation interval in each plot. The rainfall was also recorded daily.

RESULTS

Reliability of Sampling Locations

As already noted, the soil sampling locations used for routine consumptive use studies were half-way between the trunks of trees and the centres of tree squares. The reliability of these locations depended, in the first place, on how well they represented the average of the tree squares as a whole; and in the second place, on how uniform the rate of consumptive use was from one sampling location to another within the same plot.

Consumptive use tests made across the diagonals of tree squares showed considerable variability in rate of use from point to point across the square. Not only that, but the general trends with increasing distance from the tree varied considerably from orchard to orchard. The data at hand indicate the following conclusions:

1. The rate of consumptive use often varies widely from point to point throughout the root area in the soil. This is due in part to those factors that cause less consumptive use with greater depth, as noted in the first paper in this series (15). It is also due to variations in soil texture and to consequent variations in root concentration (13). Exceptionally high or exceptionally low concentrations of fibrous roots are often found scattered indiscriminately throughout the main root area. In addition, the concentration of fibrous roots has been found to vary somewhat with increasing distance from the trunk of a tree. Two examples of consumptive use across the diagonals of tree squares are presented in Table 1. The data shown represent mature-tree conditions only. It will be noted that there was considerable variability in consumptive use across the diagonals in both orchards. In some cases the variability was even greater than in these two examples. It should be noted that in Plot 54, the long consumptive-use period has served to bring the average consumptive use in the second and third feet of soil close to that in the first foot.

2. The variation in consumptive use across a tree panel depended to some extent on the age and size of the trees. This was true even though the trees were all rated as "mature". With the smaller trees, there was a

TABLE 1.—CONSUMPTIVE USE PER DAY ACROSS THE DIAGONAL OF A TREE SQUARE*

Depth	Consumptive use per day at following distances from trunk:							
	5 ft.**	10 ft.	15 ft.	21 ft.	15 ft.	10 ft.	5 ft.	Av.
feet	inches	inches	inches	inches	inches	inches	inches	inches
Plot 10, sandy soil, 8.5 days consumptive use period								
0 – 1	.055	.042	.046	.066	.073	.074	.071	.061
1 – 2	.028	.038	.025	.035	.033	.024	.042	.035
2 – 3	.035	.020	.042	.035	.025	.026	.031	.031
3 – 4	.028	.018	.028	.025	.000	.027	.015	.022
4 – 5	.051	.013	.007	.013	.009	.002	.008	.015
Total	.197	.131	.148	.184	.140	.171	.167	.164
Plot 54, heavy soil, 73 days consumptive use period								
0 – 1	.049	.046	.041	.035	.030	.038	.039	.038
1 – 2	.041	.036	.036	.038	.033	.025	.013	.031
2 – 3	.054	.045	.044	.031	.018	.009	.016	.031
3 – 4	.040	.032	.022	.033	.018	.010	.014	.024
4 – 5	.031	.029	.018	.017	.024	.004	.015	.019
5 – 6	.013	.014	.015	.007	.010	.011	.021	.013
6 – 7	.009	.005	.005	.002	.003	.013	.012	.007
Total	.237	.207	.181	.163	.136	.110	.130	.163

* Data for only two tests are shown, to illustrate the variability encountered across the diagonal of a tree square.

** The distances shown are those from the nearest tree trunk at either end of the diagonal. In both plots shown here, the trees were spaced 30 × 30 feet apart on the square.

tendency for the rate of consumptive use to decrease from the trunk out to the centre of the tree square. With the largest trees, however, the reverse held true. Observation indicated that these results were closely associated with the degree of concentration of fibrous roots; that is, these roots were most concentrated within six to eight feet of the trunk with the younger trees, and in the centres of the panels with the older trees.

3. The location used for routine sampling in these studies, that is, half-way between the trunk and the centre of the tree square, showed a rate of consumptive use that averaged about the same as that for the tree diagonals as a whole for all of the tests taken together; but not necessarily for any one test taken by itself.

As already noted, only three sampling locations were used in each plot. The question arises as to how uniform the consumptive use data were within each plot, and as to how much confidence could be placed in the plot means.

TABLE 2.—RATES OF CONSUMPTIVE USE AT INDIVIDUAL LOCATIONS WITHIN PLOTS*

Plot number	Depth	Consumptive use per day at following locations:			
		A	B	C	Av.
	feet	inches	inches	inches	inches
4	0 – 1	.068	.059	.064	.064
	1 – 2	.068	.052	.047	.056
	2 – 3	.072	.068	.043	.061
	3 – 4	.060	.044	.020	.041
	4 – 5	−.035	−.021	−.025	−.027
	5 – 6	.019	.025	.005	.016
	0 – 6	.252	.227	.154	.211
19	0 – 1	.094	.102	.061	.086
	1 – 2	.035	.077	.094	.069
	2 – 3	.065	.110	.020	.065
	3 – 4	.029	.078	.010	.039
	4 – 5	−.023	.032	.009	.006
	5 – 6	.013	.016	.009	.013
	0 – 6	.213	.415	.203	.278
45	0 – 1	.074	.093	.084	.084
	1 – 2	.041	.082	.084	.069
	2 – 3	.043	.038	.008	.030
	3 – 4	.033	.025	−.021	.012
	4 – 5	.008	.020	−.003	.008
	5 – 6	.003	.006	.000	.003
	0 – 6	.202	.264	.152	.206
47	0 – 1	.052	.048	.060	.053
	1 – 2	.056	.045	.054	.052
	2 – 3	.038	.016	.032	.029
	3 – 4	.011	.016	.026	.018
	4 – 5	.010	.012	.019	.014
	5 – 6	.000	.011	.016	.009
	0 – 6	.167	.148	.207	.175

* Data are shown for four plots only, to illustrate the variability normally encountered among the three locations within each plot.

In order to answer this question, an analysis was made of the variability within each of 33 plots. The total consumptive use values to a depth of six feet were used in making the calculations. The standard error of consumptive use was 0.124 inch per day, or 53.5 per cent of the general mean. The standard error of the plot means was $53.5/\sqrt{3} = 30.4$ per cent.

Typical consumptive use records at each of three locations within each plot are shown for four plots in Table 2. The data in this table illustrate the degree of variability normally encountered from one location to another within a plot, even when the locations sampled were all at the same distance from tree trunks.

Effects of Evaporating Power of Air on Rate of Consumptive Use

The daily rate of consumptive use was correlated with the daily rate of evaporation from an open water tank. This latter was assumed to represent as well as any other one measurement the evaporating power of the air. The coefficient of correlation obtained from 41 tests was +0.578

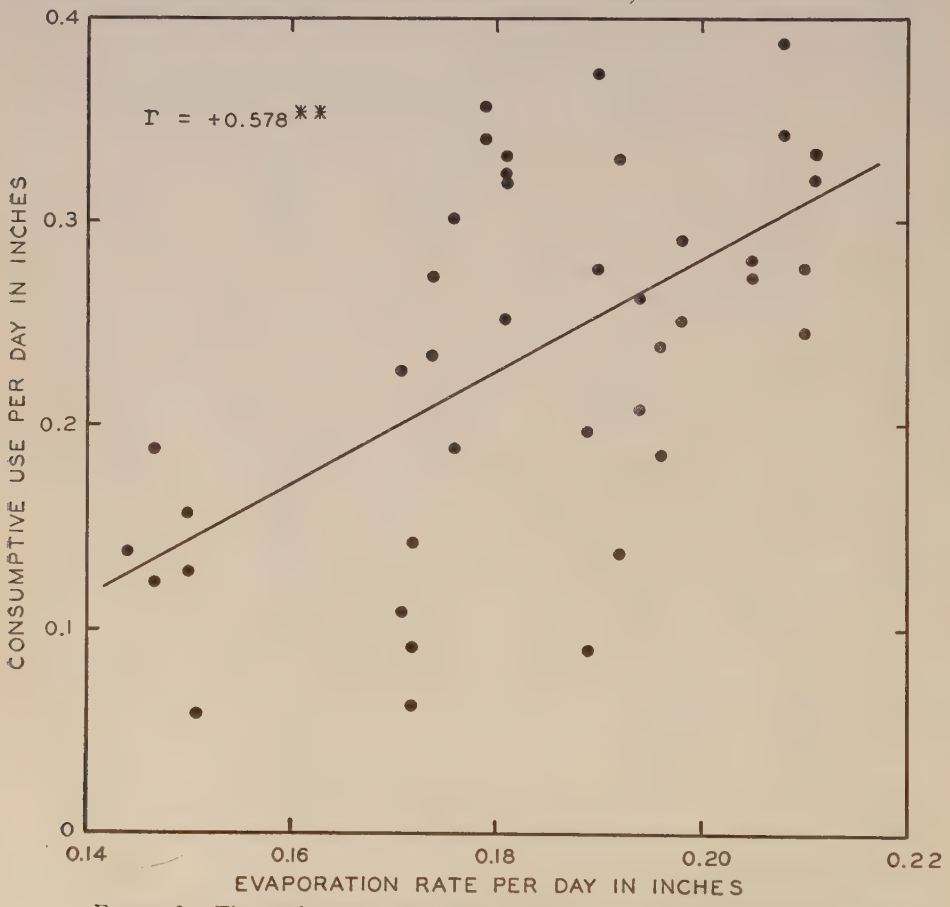


FIGURE 2. The total consumptive use per day, to a depth of 6 feet, plotted against the rate of evaporation per day from an open water tank. The regression line is shown. It has the following equation: $y = 2.87x - 0.2924$, in which y = rate of consumptive use in inches per day, and x = rate of evaporation in inches per day.

which is statistically "highly significant", having odds greater than 99 : 1. This correlation is illustrated in Figure 2, in which the daily rate of consumptive use is plotted against the daily rate of evaporation.

It will be noted in Figure 2 that there is considerable variation of the plotted points away from the regression line. This has been found to be caused partly by the errors involved in soil sampling, and partly by variability in the size of tree. Even though mature trees only were used, they still varied widely in their size per unit area of ground occupied. This resulted in a wide range in rate of consumptive use from plot to plot for any one rate of evaporation from an open water tank.

Effects of Irrigation Interval on Rate of Consumptive Use

The actual irrigation interval was usually two days longer than the consumptive use interval. For convenience, however, the consumptive use interval has been called the "irrigation interval".

The coefficient of correlation obtained between the length of irrigation interval in days and the daily rate of consumptive use was −0.383. This

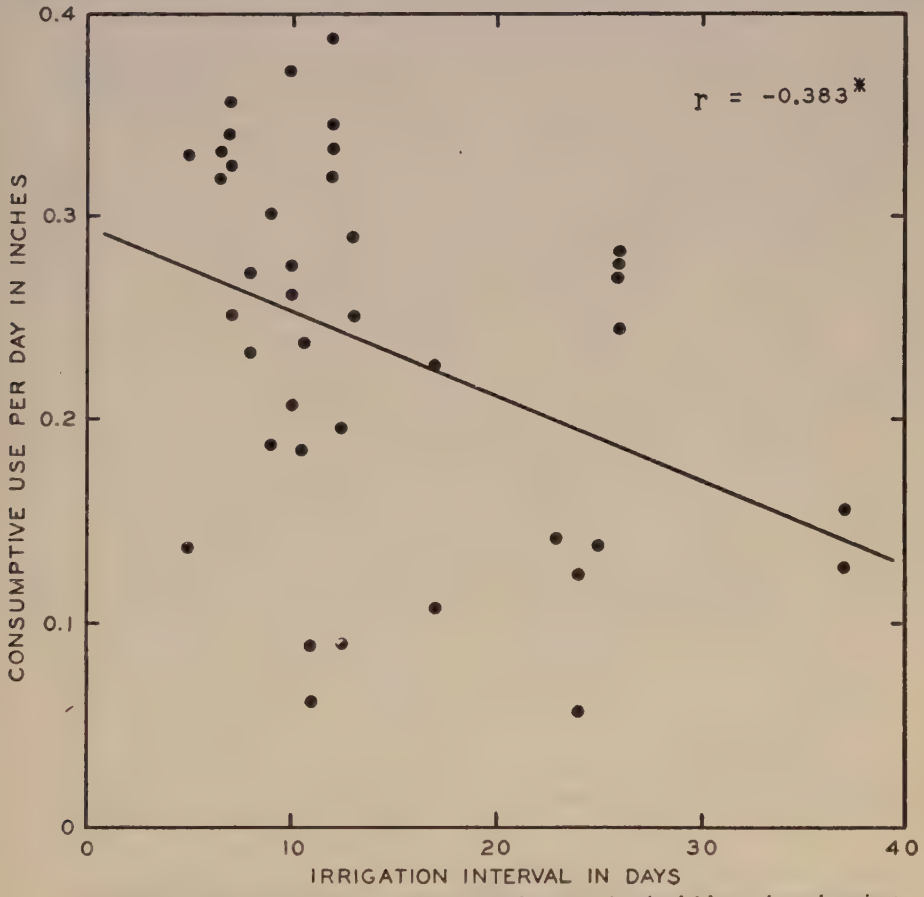


FIGURE 3. The total consumptive use per day, to a depth of 6 feet, plotted against the length of irrigation interval. The regression line is shown. It has the following equation: $y = .2957 - 0.00416x$, in which y = rate of consumptive use in inches per day, and x = length of irrigation interval in days.

is statistically "significant", having odds greater than 19 : 1 and less than 99 : 1. This means that an increase in length of irrigation interval was accompanied by a reduction in rate of consumptive use. On the whole, the longer intervals used by growers were on heavier soils, and the shorter intervals on lighter soils. The correlation is illustrated in Figure 3, in which the daily rate of consumptive use is plotted against the irrigation interval in days. It will be noted that the points diverge from the regression line even more widely than they do in Figure 2.

Effects of Irrigation Interval on Rate of Evaporation from Evaporimeter

The rate of evaporation from an open water tank has proved to be much greater during the heat of the summer than during the cooler part of the summer. The hottest and driest days usually last for only a few days at a time. As a result, the average rate of evaporation per day during any irrigation interval is affected by the length of this interval. For example, the hottest and driest 10-day period during the summer has a higher average rate of evaporation per day than has the hottest and driest 20-day period.

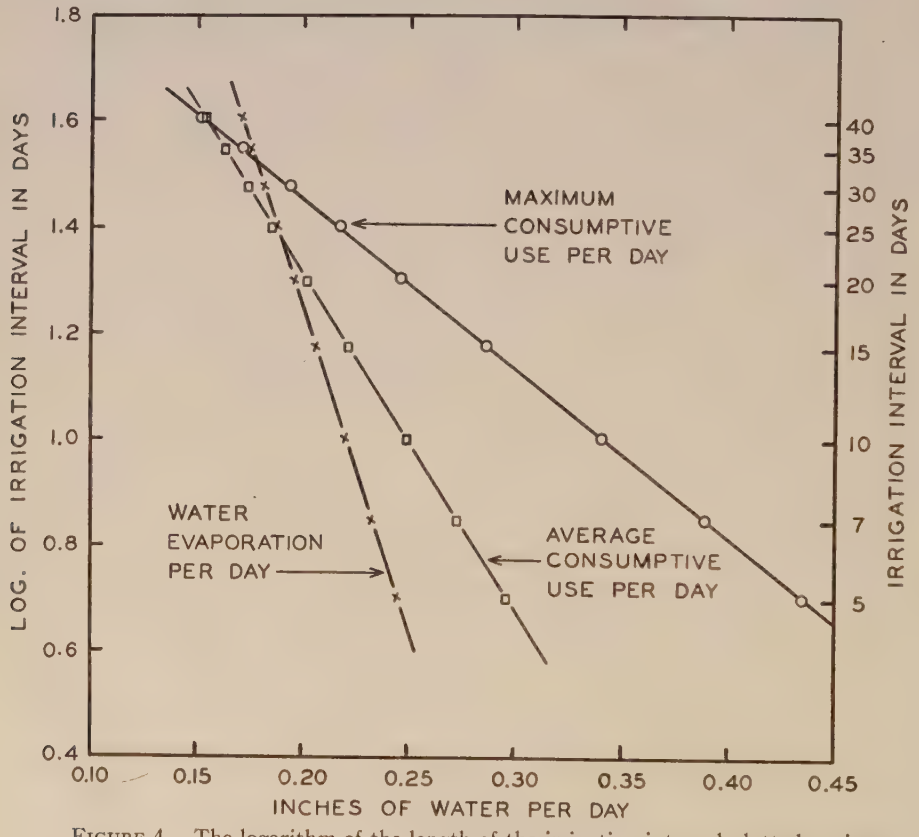


FIGURE 4. The logarithm of the length of the irrigation interval plotted against (1) the maximum rate of evaporation of water from an open tank, (2) the average consumptive use of water, and (3) the maximum consumptive use of water. Only the regression line for each is shown.

The data obtained from each evaporimeter in 1950 were examined to determine the 5-day period having the highest rate of evaporation, and the average daily rate of evaporation for this period was calculated. The same was then done for periods of 7, 10, 15, 20, 25, 30 and 40 days. The rates thus calculated for each period were averaged for the six evaporimeters that had been placed in different districts in the Okanagan Valley. The values obtained are assumed to represent the average maximum rates of evaporation for different lengths of irrigation interval in 1950. The results are charted in Figure 4, in which the maximum evaporation per day is plotted against the logarithm of the length of interval in days, in the curve entitled "water evaporation per day". It will be seen that this curve is a straight line and has a negative coefficient. The equation representing this curve was as follows:

$$y = 0.3025 - 0.0822 \log x,$$

in which y = average rate of evaporation per day, during the hottest and driest irrigation interval,

and x = irrigation interval in days.

This equation indicates that as the irrigation interval was increased by a constant step (say 5 days) from 5 days to 40 days, the rate of evaporation per day decreased in geometrical proportion.

The results of this study indicate the possibility that since there was such a close relationship between the rate of evaporation and the rate of consumptive use, there might also be a straight-line relationship between the rate of consumptive use and the logarithm of the length of the irrigation interval. The equation representing this relationship in 1950 was as follows:

$$y = 0.411 - 0.160 \log x,$$

in which y = average consumptive use per day in inches,

and x = irrigation interval in days.

This equation is represented in Figure 4 by the line entitled "average consumptive use per day". It appears to represent the trend better than does the ordinary regression line shown in Figure 3.

Combined Effects of Evaporating Power of Air and Irrigation Interval on Rate of Consumptive Use

As noted above, the longer the irrigation interval, the less was the daily rate of evaporation during that interval which occurred at the time of greatest evaporation. Because of this relationship, the length of irrigation interval has been found to have an indirect effect on average daily rate of consumptive use during the hottest and driest part of the season, that is, on the maximum daily rate of consumptive use for each length of interval. In the 1950 investigations, not over half of the tests were actually conducted during the hottest part of the season. The "average consumptive use per day" curve in Figure 4 represents the trend of the results actually obtained. The question is, what would have been the trend if all of the tests had been conducted during the hottest part of the summer?

In order to answer this question, it was necessary first of all to calculate the equation involving the regression of rate of consumptive use on both the rate of evaporation per day and the length of irrigation interval. This equation was as follows:

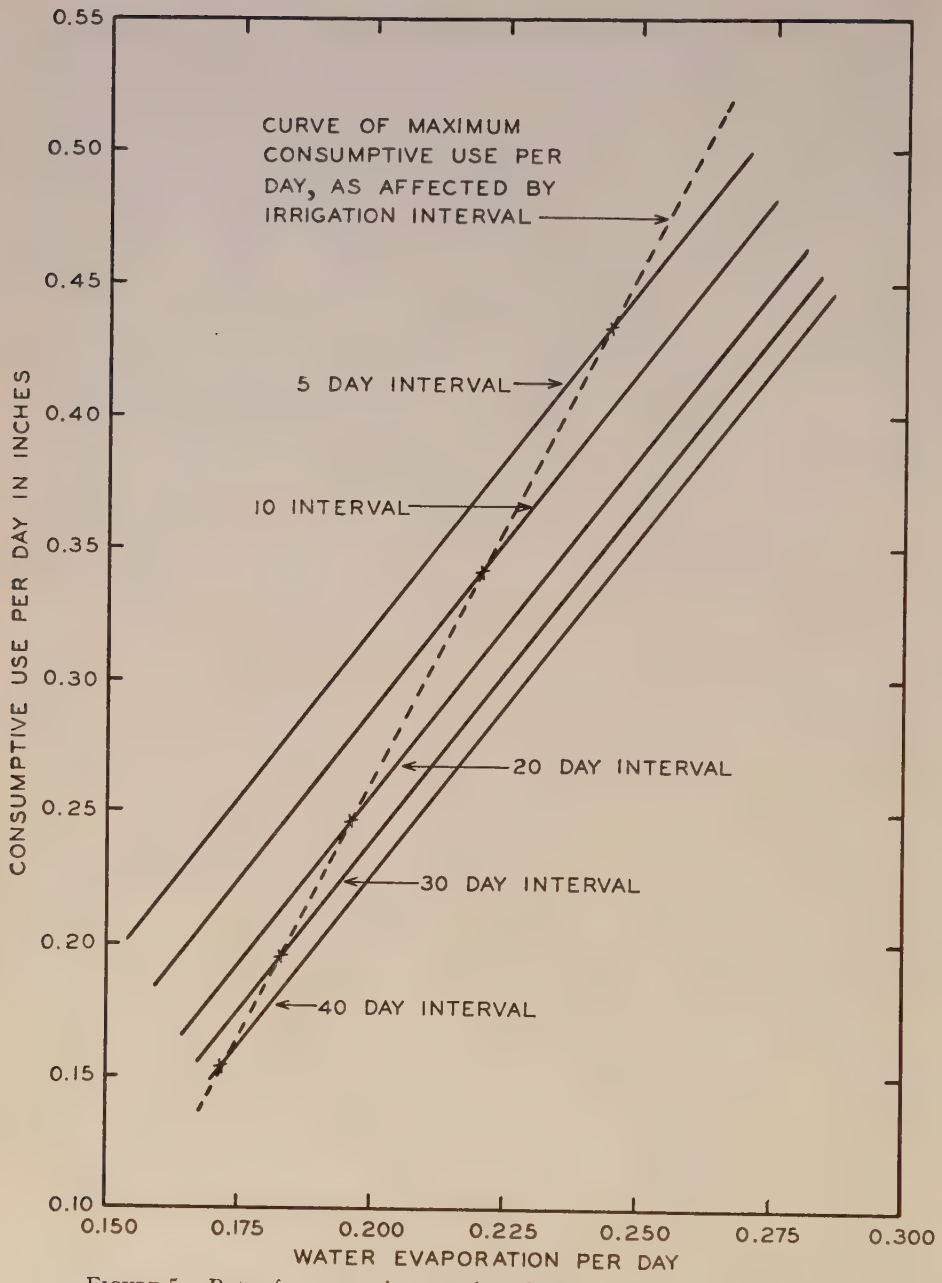


FIGURE 5. Rate of consumptive use plotted against the rate of evaporation from an open water tank. Separate regression lines are shown for irrigation intervals of 5, 10, 20, 30 and 40 days. The curve of maximum consumptive use per day, as affected by the length of the irrigation interval, is also shown.

$$y = -0.122 + 2.57x - 0.1044 \log z,$$

in which y = consumptive use per day, in inches,
x = evaporation per day from evaporimeter, in inches,
and z = irrigation interval in days.

If for x in this equation we substitute the values for the maximum evaporation per day for each irrigation interval (as in Figure 4), we obtain the maximum consumptive use values shown in Table 3. These same values are charted against the logarithm of the irrigation interval in Figure 4, under the heading "maximum consumptive use per day".

TABLE 3.—EFFECTS OF LENGTH OF IRRIGATION INTERVAL ON MAXIMUM CONSUMPTIVE USE PER DAY

Irrigation interval	Maximum evaporation per day	Maximum consumptive use per day
days	inches	inches
5	0.245	0.435
7	0.233	0.395
10	0.221	0.341
15	0.207	0.287
20	0.197	0.248
25	0.189	0.218
30	0.183	0.194
35	0.177	0.172
40	0.172	0.153

By using a constant irrigation interval in this equation and varying the rate of evaporation, a curve can be obtained representing the relation between rate of evaporation per day and rate of consumptive use per day. This can be done for each length of irrigation interval. A series of such curves is shown in Figure 5. On each of these curves, that point has been noted that represents the maximum rate of evaporation for the interval concerned. These points have been joined together to give the curve entitled "curve of maximum consumptive use per day, as affected by irrigation interval". This curve is also a straight line. It provides the same maximum consumptive use values as shown in Table 3, and as indicated in Figure 4 along the line "maximum consumptive use per day".

In the equation noted above, relating the rate of consumptive use to both the rate of evaporation and the length of the irrigation interval, the factor attached to the rate of evaporation was +2.57. This means that on the average, each change in the rate of evaporation was accompanied by 2.57 times as much change in the rate of consumptive use. In view of the wide variability of the points in Figure 2, this coefficient 2.57 cannot be considered accurate. Indications are, however, that the effects of the evaporating power of the air on consumptive use were much greater than proportional to the rate of evaporation from an open water tank.

DISCUSSION

Reliability of Data

In a field survey type of investigation such as that reported in this paper, it is not always possible to sample the whole plot adequately in every

orchard. Some selection of sampling locations appears advisable and even necessary.

As indicated in the results reported above, the reliability of the sampling position used in this investigation (half-way between the trunk and the centre of the tree square) depends to some extent on the age of the trees. In previous work (13), it was found that as the trees grow older the concentration of fibrous roots farther out in the panel increases progressively. This has a direct effect on the rate of consumptive use of water from the soil. The evidence available thus far indicates that with younger trees, whose root systems show little or no overlapping from tree to tree, the rate of consumptive use is greatest within four feet or so from the trunk and decreases with greater distance from the trunk. As the trees grow, however, and the roots from adjacent trees meet and then overlap more and more, the rate of consumptive use increases greatly farther out from the tree and may even decrease somewhat closer to the trunk. Finally, the rate in the centre of the panel may become greater than at any other point. With mature trees, the sampling location used in this investigation appears to represent reasonably well the tree square as a whole.

Only three sampling locations were used in each plot. The coefficient of variability of the consumptive use records from individual locations was 53.5 per cent, indicating very high variability. The coefficient for plot averages was 30.4 per cent. As a result, the records from any one plot cannot be considered sufficiently reliable for individual plot comparisons. To be considered reliable for this purpose, more sampling locations would be needed, possibly in a grid pattern across the tree square. The records obtained should, however, be reasonably suitable for the type of correlation study made in this investigation.

The amounts of irrigation water actually applied by the growers to the plots used in this investigation were measured both before and after each consumptive use interval. The results of this phase of the work have not been reported in this paper. They do reveal a weakness in the consumptive use data, however, and this should be mentioned. In most plots, the amounts of water applied by the growers were greater than necessary. In some plots, however, the amounts applied appeared to be just sufficient to maintain adequate moisture in the soil. The monthly rate of application of irrigation water, where just sufficient water was applied, was found to average about the same as, or even lower than, the monthly rate of consumptive use. It might be concluded from this that the efficiency of irrigation in these orchards was 100 per cent. Studies that have been made on soil moisture contents before and after irrigation, however, indicate that this conclusion would be erroneous. It appears obvious that on the average, the consumptive use values obtained in this investigation were somewhat higher than they should have been. Just why, has not been determined. Whatever bias there may have been in the data, however, appears to have been reasonably consistent. This means that the major findings, dealing with the effects of certain factors on the rate of consumptive use, can still be considered valid.

Evaporation from an Open Tank

No attempt was made, in this investigation, to study those meteorological factors affecting the rate of evaporation of water from an open tank. It is quite possible that some of these factors might have differential effects on the rates of evaporation and transpiration. For example, a strong wind might increase the rate of evaporation but decrease the rate of transpiration. The assumption has been made in this paper, however, that the rate of evaporation from an open tank is as good a single measure as would be any one of those meteorological factors affecting both the rate of transpiration and the rate of evaporation from the surface of the soil. Whether this assumption is valid has not been determined. If it is, it will only hold true under conditions of constant leaf area per acre and uniform leaf activity. Standards established during the heat of the summer will obviously not apply early in the season before the leaves are fully formed, nor late in the season when they are maturing or have fallen.

Equations were presented above, relating the rate of evaporation from an open water tank to the rate of consumptive use. These equations indicated that an increase in rate of evaporation was accompanied by a considerably greater increase in rate of consumptive use. Just how this effect was brought about has not been determined. It appears reasonable to assume that the major effect of variations in the evaporating power of the air was on the rate of transpiration. There may also, however, have been a considerable effect on the rate of evaporation from the surface of the soil. It was not found possible in this investigation to determine these effects separately.

Length of Irrigation Interval

It was found in this investigation that the shorter the irrigation interval, the greater was the daily average rate of consumptive use of water. This can be attributed largely to the greater evaporation of water from the surface of the soil. Following an irrigation, all of the soil profile (including the surface soil) contains moisture at the field capacity. Evaporation from the surface, therefore, is rapid. As the surface soil dries out the rate of evaporation lessens progressively. During the first 5 days, the rate of evaporation is greater than in the next five days; and during the first 10 days it is greater than in the next 10 days. Thus the average rate of evaporation is less during a 20-day irrigation interval than it is during a 10-day interval.

To some extent, the above tendency is offset by the effects of differences in soil textures. On the whole, the heavier the soil the longer is the safe irrigation interval. In the tests reported in this paper, the soil in the long-interval plots was heavier (with one or two exceptions) than the soil in the short-interval plots. A heavy soil holds more water in the surface six inches than does a sandy soil; and as a result, the rate of evaporation from the surface averages higher during any one length of irrigation interval than it does with a sandy soil (10). This effect of soil texture, however, is not great enough to offset the negative relationship noted above between length of irrigation interval and average daily rate of evaporation from the surface of the soil.

Growers do not always irrigate at the correct irrigation interval. A practice that is sometimes encountered in the Okanagan Valley is the application of irrigation water much more frequently than necessary. Thus a heavy soil, with a high capacity for sustained evaporation, may be irrigated frequently and thus kept relatively wet at the surface. In such a case the daily rate of evaporation averages higher than with a sandy soil that is irrigated at the same periods. By way of example, Plot 20, a heavy soil that could have lasted 40 days between irrigations was irrigated every 10 days and had a rate of consumptive use much higher than any of the plots in sandy soils that were irrigated every 10 days. This is indicated in the data presented in the first paper of this series (15). Thus, unnecessarily frequent irrigation causes wastage of water by increased evaporation from the surface of the soil.

Wastage of irrigation water occurs in other ways as well. With furrow irrigation, for example, there is loss of water into the deeper subsoil and over the ends of the furrows. With sprinkler irrigation, there is loss by evaporation from the drops of water while they are in the air; and even with careful sprinkling, there is some loss into the subsoil. Thus there is wastage of water in various ways during and following each irrigation; and some of this wastage is unavoidable. This has a very definite bearing on the amount of water required for irrigation. The more frequent the irrigation, the greater is the wastage of water during each month of irrigation. Where frequent irrigation is necessary, therefore, such as with sandy soils, more irrigation water is required than where frequent irrigation is not necessary.

The question arises as to whether the length of irrigation interval affects the rate of transpiration. It has been shown by other investigators (1, 4) that if any major part of the soil surrounding the absorbing roots is allowed to dry down to the permanent wilting point, transpiration by tree leaves is reduced. This is confirmed in unpublished work by the author. Assuming this to be so, it would follow that if longer irrigation intervals caused greater relative drying of the soil, then the rate of transpiration might be reduced more than with shorter intervals. This could well happen where long intervals and short intervals were being compared on soils similar in texture and depth. Long intervals are, however, normally associated with heavy soils having a large reservoir of available water, whereas short intervals are normally associated with light soils having a small reservoir of moisture; so that theoretically at least, the texture of the soil should have little effect on the relative degree of drying. In this investigation, records of the relative degree of drying were obtained in only two plots. However, observational evidence indicates that on the whole, the lighter soils in the short-interval plots were allowed to dry out relatively more between irrigations than were the heavier soils in the long-interval plots. This should have had the opposite effect on consumptive use from that actually found. It is doubtful if variation in the length of irrigation interval had any marked direct effect on the rate of transpiration.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of the following men in conducting the field and laboratory work involved in this investigation:

R. G. Garry, British Columbia Department of Agriculture, Kelowna, B.C.; S. D. Dyson, Experimental Substation, East Kelowna, B.C.; M. Power, D. Faris and D. S. Stevenson, Experimental Station, Summerland, B.C.

REFERENCES

1. Aldrich, W. W., and R. A. Work. Evaporating power of the air and top-root ratio in relation to rate of pear fruit enlargement. Proc. Amer. Soc. Hort. Sci. 32 : 115–123. 1934.
2. Aldrich, W. W., R. A. Work, and M. R. Lewis. Pear root concentration in relation to soil-moisture extraction in heavy clay soil. J. Agr. Res. 50 : 975–988. 1935.
3. Israelsen, O. W. Water-application efficiencies in irrigation and soil conservation. Agr. Eng. 20 : 423–425. 1939.
4. Lewis, M. R., R. A. Work, and W. W. Aldrich. Studies of the irrigation of pear orchards on heavy soil near Medford, Oregon. U.S. Dept. Agr. Bull. 432. 1934.
5. Meyer, B. S., and D. B. Anderson. Plant physiology. D. Van Nostrand Co. Inc., New York. 1939.
6. Miller, E. C. Plant physiology. McGraw-Hill Book Co. Inc., New York. 1931.
7. Pair, C. H., and W. D. Criddle. Peak consumptive use for irrigation systems. *Private correspondence.* 1951.
8. Powers, W. L., and M. R. Lewis. Irrigation requirement of arable Oregon soils. Oregon Agr. Exp. Sta. Bull. 394. 1941.
9. Schuster, C. E., and R. E. Stephenson. Soil moisture, root distribution and aeration as factors in nut production in Western Oregon. Oregon Agr. Exp. Sta. Bull. 372. 1940.
10. Smith, G. E. P. Use and waste of irrigation water. Arizona Agr. Exp. Sta. Bull. 101. 1925.
11. Smith, G. E. P., A. E. Kinnison, and A. G. Carns. Irrigation investigations in young grapefruit orchards on the Yuma Mesa. Arizona Agr. Exp. Sta. Tech. Bull. 37. 1931.
12. Veihmeyer, F. J., and A. H. Hendrickson. Soil moisture as an indication of root distribution in deciduous orchards. Plant Physiol. 13 : 169–177. 1938.
13. Wilcox, J. C., and A. T. Knight. Some factors affecting apple yields in the Okanagan Valley. III. Root distribution. Sci. Agr. 25 : 760–775. 1945.
14. Wilcox, J. C. Sprinkler irrigation of orchards in British Columbia. Canada Dept. of Agr. Pub. 797. 1947.
15. Wilcox, J. C., and J. L. Mason. Consumptive use of water in orchard soils. I. Effects of soil depth. Can. J. Agr. Sci: 101–115. 1953.

A STUDY OF THE CAROTENE AND CRUDE PROTEIN CONTENT OF ORCHARDGRASS (*DACTYLIS GLOMERATA L.*)

II. HERITABLE VARIATION[1]

M. F. CLARKE[2]

Experimental Station, Agassiz, B.C.

[Received for publication September 5, 1952]

ABSTRACT

In order to obtain evidence of heritable variation in the carotene and crude protein content of orchardgrass, a selected group of plants from a combining ability experiment were analysed. The categories of material analysed included open-pollinated clones and fifth generation inbreds (I_5). The two classes of clones were crossed on two tester plants, one an unrelated open-pollinated clone of low carotene and protein content and the other an unrelated I_5 clone with a high analysis for the two constituents. In addition, a limited number of singlecrosses between open-pollinated clones were examined.

Significant differences in content of carotene and crude protein were obtained for both the parents and the I_5 clones derived from them. In testcrosses with open-pollinated clones a significant tester effect was obtained for both carotene and protein. The mean tester effect in crosses using I_5 clones lacked significance although slightly higher values were apparent for the high tester. The means of individual crosses with both testers showed highly significant differences for carotene and protein. The implications arising out of the data obtained were discussed.

In a companion paper (3) data were presented on the variations in carotene and crude protein content of orchardgrass that could be accounted for by (*a*) stage of growth, (*b*) cutting management and (*c*) clones. It was found that the differences in carotene and crude protein which could be ascribed to stage of growth and management were of considerable magnitude. Differences between the clones studied, although of small magnitude, were statistically significant for both carotene and crude protein.

Information on the heritable variation in carotene protein in grasses is limited. Pickett (6) found highly significant differences in carotene and protein content among the plants of 20 first-generation inbred lines of bromegrass. Tsiang (8), also working with bromegrass, provided partial evidence of heritable variation in carotene. He obtained a significant value of +0.76 for parental clones and their one-year selfed progenies. No attempt was made, however, to follow the inheritance of this constituent further.

Among other crops, Kohler and his co-workers (4) have achieved considerable success in breeding for higher carotene content in tomatoes. Millar and Covington (5) provided evidence that selection and breeding for higher carotene content was possible in sweet potatoes. Aurand *et al.* (2) studied the influence of heredity upon the carotene content of corn and reported that genetic constitution may be responsible in a large measure for carotene content.

MATERIALS AND METHODS

The data reported in this paper were obtained by sampling orchardgrass plants in a combining ability study located at the United States

[1] Contribution from the Department of Agronomy, The Pennsylvania State College; the United States Regional Pasture Research Laboratory, State College, Pennsylvania; and the Experimental Farms Service, Canada Department of Agriculture. Authorized for publication on June 25, 1952, as paper No. 1740 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Senior Agronomist, Canada Department of Agriculture, Experimental Farm, Agassiz, B.C.

Department of Agriculture Regional Pasture Research Laboratory, State College, Pennsylvania. The object of the original experiment was to obtain information on the effect of inbreeding on combining ability. The plant material sampled included 14 open-pollinated clones and 12 clones selected from the fifth-generation inbred progenies (I_5) of 7 of the open-pollinated clones. The two classes of clones were crossed on two tester plants, one a selected open-pollinated clone and the other an unrelated I_5 clone. The testers were the main plots and the clones were the subplots for both the open-pollinated and I_5 clones. In addition, six single crosses of four of the open-pollinated clones were included in the same planting as the parents X testers series.

All plots comprised 10 spaced plants. Three replications were sampled for each test-cross series involving both classes of clones. The data for the single crosses involving open-pollinated clones were based on six replications.

Sampling was carried out during the summer of 1950 at the vegetative stage or when the plants averaged 6 inches in height. In order to provide actively growing tissue of uniform age the plants were first clipped back to $1\frac{1}{2}$ inches above ground level and the recovery growth was sampled for

TABLE 1.—MEAN CAROTENE AND CRUDE PROTEIN CONTENT OF PARENTAL, I_5 CLONES AND TESTER PLANTS OF ORCHARD GRASS

Open-pollinated Clone	Carotene Mgm./100 gm.	Protein Per cent	I_5 Clone	Carotene Mgm./100 gm.	Protein Per cent
1-C*	50.0	30.3	1–3	34.4	25.1
2–C*	38.4	28.2	1–6	34.6	27.7
3–C	28.5	27.5	2–1	53.2	27.4
4–C*	41.5	29.0	2–4	31.2	25.4
7-C*	41.7	27.9	4–1	51.3	28.0
9-C	38.2	25.7	4–3	29.8	26.0
10-C	25.3	24.1	7–2	30.7	25.4
12-C*	32.8	26.7	7–3	27.7	23.8
13-C	38.8	25.0	12–6	36.1	25.6
14-C*	35.6	26.8	12–12	35.9	26.3
15-C*	28.1	25.4	14–2	32.2	23.9
16-C	42.8	28.5	15–2	37.4	28.5
19-C	36.1	27.1			
5-C	28.5	25.7			
Tester Plants					
MI-13 (O.P. Clone)	29.5	24.7			
426 (I_5 (Clone)	51.4	29.3			

* Clones from which I_5 clones have been derived, 1-C parent of 1–3 and 1–6, etc.

L.S.D. at	Between Means of:			
	O.P. Clones and Testers		I_5 Clones	
	Carotene	Protein	Carotene	Protein
P = 0.05	1.3	0.2	1.7	0.4
P = 0.01	1.8	0.3	2.3	0.5

analysis. The preparation of samples for analysis was according to the procedure previously outlined by the author (3).

Extraction of carotene was according to the Blendor extraction method of Zscheile and Whitmore (9). Carotene determinations on the extracts were made according to the tentative A.O.A.C. method (1), modified by the use of celite-magnesium oxide 3 : 1 as the chromatographic absorbent.

Crude protein content (N × 6.25) was determined according to the Kjeldahl-Gunning-Arnold method as modified by the A.O.A.C. (1).

RESULTS

The mean values for carotene and crude protein for the classes of material analysed are listed in Table 1. A wide range in carotene content

TABLE 2.—SUMMARY OF MEAN CAROTENE AND CRUDE PROTEIN CONTENTS FOR PROGENIES OF 10 OPEN-POLLINATED CLONES OF ORCHARDGRASS IN TWO TEST-CROSSES

Carotene, mgm. per 100 gm. Dry Matter				Per Cent Protein, Dry Weight Basis			
O.P. clones	Low tester MI-13 (29.5) *	High tester 426 (51.4)	Means of means of crosses	O.P. clones	Low tester MI-13 (24.7)*	High tester 426 (29.3)	Means of means of crosses
15-C (28.1)*	33.3	38.2	35.7	13-C (25.0)*	24.8	25.1	24.9
5-C (28.5)	27.7	36.3	32.0	15-C (25.4)	26.2	27.5	26.9
12-C (32.8)	32.9	37.3	35.1	9-C (25.7)	24.7	26.9	25.8
14-C (35.6)	38.4	36.1	37.3	5-C (25.7)	24.7	26.5	25.6
19-C (36.1)	34.3	40.1	37.2	12-C (26.7)	26.3	26.3	26.3
9-C (38.2)	40.6	47.5	44.0	14-C (26.9)	26.5	26.9	26.7
13-C (38.8)	31.3	33.1	32.2	19-C (27.1)	26.3	26.1	26.2
4-C (41.5)	34.3	43.5	38.9	16-C (28.5)	26.3	27.4	26.9
16-C (42.7)	34.8	47.1	40.9	4-C (29.1)	27.1	27.7	27.4
1-C (50.0)	30.3	41.7	36.0	1-C (30.3)	27.6	28.1	27.9
Means for testers	33.8	40.1			26.1	26.8	

* Numbers in brackets are parental values for carotene and protein.

L.S.D. at	Between Means of:			
	Means of Crosses		Testers (Foot of Table)	
	Carotene	Protein	Carotene	Protein
P = 0.05	2.7	1.1	1.2	0.5
P = 0.01	3.6	1.5	1.6	0.7

TEST OF ASSOCIATION BETWEEN MEANS OF PARENTS AND MEANS OF PROGENY

	Carotene (*D.F.* = 9)		*Protein* (*D.F.* = 9)	
Low Tester Series	$x^2 = 5.9$	$P = 0.8 - 0.7$	$x^2 = 0.1$	$P = 0.99$
High Tester Series	$x^2 = 7.7$	$P = 0.7 - 0.5$	$x^2 = 0.8$	$P = 0.99$

is indicated for both open-pollinated clones and the I_5 clones derived from them. The variation in crude protein was significant in both categories of material. The unrelated plants selected for test crosses also showed marked differences, the open-pollinated clone MI-13 being low in carotene and protein and the I_5 clone being high in both constituents.

It will be noted that the 12 I_5 clones tested have been derived from only 7 of the 14 open-pollinated clones examined. In this connection it is of interest that 7 of the inbreds (I_5) have shown a higher mean carotene content

TABLE 3.—SUMMARY OF MEAN CAROTENE AND CRUDE PROTEIN CONTENTS FOR PROGENIES OF TWELVE INBRED (I_5) CLONES OF ORCHARDGRASS IN TWO TEST-CROSSES

Carotene, mgm. per 100 gm. Dry Matter				Per Cent Protein, Dry Weight Basis			
I_5 Clones	Low tester MI-13 (29.5)*	High tester 426 (51.4)	Means of means of crosses	I_5 Clones	Low tester MI-13 (24.7)*	High tester 426 (29.3)	Means of means of crosses
7–3 (27.7)*	34.7	32.1	33.1	7–3 (23.8)*	24.3	26.6	25.5
4–3 (29.8)	36.9	34.6	35.7	14–2 (23.9)	27.6	28.5	28.1
7–2)30.7)	34.9	33.7	34.3	1–3 (25.1)	30.5	25.9	28.2
2–4 (31.2)	34.6	42.4	38.5	7.2 (25.4)	26.3	28.5	27.4
14–2 (32.2)	38.2	37.4	37.8	2–4 (25.5)	26.4	27.5	26 9
1–3 (34.4)	31.9	37.3	34.6	12–6 (25.6)	25.9	28.0	26.9
1–6 (34.6)	34.8	36.5	35.6	4–3 (26.0)	26.0	26.6	26.3
12–12 (35.9)	40.7	35.2	37.9	12–12 (26.3)	25.6	28.8	27.2
12–6 (36.1)	38.1	37.8	37.9	2–1 (27.4)	27.3	27.9	27.6
15–2 (37.4)	37.3	37.3	37.3	1–6 (27.7)	27.5	28.5	28.0
4–1 (51.3)	34.9	44.5	39.7	4–1 (27.8)	26.0	27.2	26.6
2–1 (53.2)	35.7	43.2	39.5	15–2 (28.5)	26.7	27.9	27.3
Means for testers	36.0	37.7			26.3	27.7	

* *Numbers in brackets are parental values for carotene and protein.*

L.S.D. at	Between Means of:			
	Means of Crosses		Testers (Foot of Table)	
	Carotene	Protein	Carotene	Protein
P = 0.05	3.0	1.3	—	—
P = 0.01	4.1	1.7	—	—

TEST OF ASSOCIATION BETWEEN MEANS OF PARENTS AND MEANS OF PROGENY

	Carotene (*D.F.* = *11*)		*Protein* (*D.F.* = *11*)	
Low Tester Series	$x^2 = 10.9$	$P = 0.5 - 0.3$	$x^2 = 2.2$	$P = 0.99$
High Tester Series	$x^2 = 14.9$	$P = 0.2 - 0.1$	$x^2 = 0.4$	$P = 0.99$

than the parents from which they were derived. The data for protein show only one inbred, namely 15–2, to be higher than its parent (15-C).

The results obtained from two test-crosses involving the open-pollinated clones are summarized in Table 2. Highly significant differences for both carotene and crude protein have been obtained between the means of the clones in the two test-crosses. In addition the mean carotene and crude protein contents of all crosses on the high tester plant (426) were significantly better than those obtained with the low tester plant (MI-13). The general agreement between the test-cross values for carotene and the means of the parents, as determined by the Chi-square test, was quite close with a slightly closer association being shown for crosses involving the low tester. In the case of crude protein the test-cross values show an extremely close association with the parental means.

The behaviour of the I_5 clones in the two test-crosses was similar to that of the open-pollinated clones as indicated by the data presented in Table 3. The differences between means of the I_5 clones for crosses on both testers were again highly significant for the two constituents examined. The effect of the testers was not significant, although slightly higher mean values were obtained for the crosses using the high tester. The x^2 test for the association of the mean value of crosses with the means of the parents indicated satisfactory agreement in the case of carotene. The degree of association was somewhat less than that obtained in crosses involving the open-pollinated clones. The data for protein again indicated a very close association between parental and crossed values.

TABLE 4.—SUMMARY OF MEAN CAROTENE AND CRUDE PROTEIN CONTENTS FOR PROGENIES OF FOUR OPEN-POLLINATED CLONES OF ORCHARDGRASS IN SIX SINGLE CROSSES

Cross	Carotene Mgm./100 gm.	Protein Per cent
14-C × 1-C	34.6	27.6
14-C × 19-C	35.8	27.1
5-C × 14-C	37.4	25.8
1-C × 19-C	39.1	27.5
19-C × 5-C	43.5	27.1
5-C × 1-C	47.6	26.7
Mean of Parents		
5-C	28.5	25.7
14-C	35.6	26.9
19-C	36.1	27.1
1-C	50.6	30.3

L.S.D. at	Between Means of:			
	Crosses		Parents	
	Carotene	Protein	Carotene	Protein
P = 0.05	3.9	1.1	1.3	0.2
P = 0.01	5.3	—	1.8	0.3

TABLE 5.—CORRELATION COEFFICIENTS (r) BETWEEN CAROTENE AND CRUDE PROTEIN FOR THE POPULATIONS OF ORCHARDGRASS MATERIAL

Population	D.F.	r
Open-pollinated clones	18	+0.2068
I_5 clones	22	+0.4348*
O.P. clones × MI-13	28	+0.8550**
I_5 clones × MI-13	34	+0.1005
O.P. clones × 426	28	+0.8110**
I_5 clones × 426	34	+0.5588**
Single crosses O.P. clones	34	+0.0377

* Significant P = 0.05 or less.
** Significant P = 0.01 or less.

Table 4 shows the mean carotene and crude protein content for the F_1 progenies of six single crosses among four of the open-pollinated clones. The single cross values for carotene indicated marked differences. The clone 5-C appeared to be a particularly good combiner for carotene, although its initial content was low. The clone 1-C with the highest parental value for carotene did not show a high mean carotene content in the progeny of two of its single crosses. When 1-C was crossed with 5-C, however, a value approaching the high parent was obtained. The protein contents of the single cross progenies were not in close agreement with the results for carotene. However, on the basis of the required difference only two classes of protein level were indicated for the means of the single crosses. The protein values for the single cross parents differed significantly, although the magnitude of the difference was small.

In order to check on the relationship between carotene and crude protein, simple correlations were calculated for the two constituents for the various categories of orchardgrass material. These are listed in Table 5. In all instances the correlations were positive. Particularly high r values were obtained for the crosses of open-pollinated clones on the tester MI-13 and for both classes of clones crossed to the tester 426. The correlation was low in the open-pollinated clones per se and significant in the inbred (I_5) group. The single crosses involving 4 open-pollinated clones showed a very low correlation for carotene and crude protein. The reason for this was partially explained by the F_1 values for the two constituents as shown in Table 4, where marked fluctuations were shown for carotene with only slight variation being indicated for protein.

DISCUSSION

The sampling data for the combining ability study with orchardgrass have established that significant differences exist for carotene and protein in the parental plants and the fifth generation inbred clones derived from them. Since the tester plants used for top-crossing varied significantly in composition for the two constituents studied, a basis is provided for the discussion of heritable behaviour for carotene and protein.

In all instances the data were for the means of populations obtained following crossing. No attempt was made to establish the variability within populations. When the open-pollinated clones were crossed on the high tester plant (426) the mean of all crosses was significantly greater for both carotene and protein than the corresponding values for crosses with the low tester(MI-13). This of itself could be regarded as evidence of the ability of the plant 426 to transmit its superior carotene and crude protein content. The test-cross data for the I_5 clones failed to provide a significant tester effect, although a slightly higher mean was obtained for crosses using the high tester (426).

For both categories of clones the F_1 means of individual crosses with the tester plants showed highly significant differences. In no instance were the means of the crosses greater than the mean of the high parent when the high value tester plant was used. For crosses on the low tester (MI-13) carotene content was higher than the high parent for eight of the ten open-pollinated clones. In the case of protein, five of the ten crosses on the low tester were higher than the means of the high parent. Among the crosses of I_5 clones on MI-13 four instances of F_1 means in excess of the high parent were obtained for carotene and in only one case for protein. It is of interest to note that, wherever heterosis has been indicated, the high parent was invariably of low rank in respect to carotene or protein. For all cases in which a high value carotene parent was used the means fell in the range between "no dominance" and "partial dominance" as defined by Powers (7). Considering all categories of crosses the data for protein tended toward lack of phenotypic dominance although partial dominance of high or low was indicated in a few cases. Considering the differences between open-pollinated parental clones and the I_5 lines derived from them it would appear that genetic factors conditioning carotene and crude protein in the parental material have segregated. The fact that certain I_5 clones were higher in carotene suggests that inbreeding has resulted in homozygosity in certain loci which affect carotene. This also can be interpreted as evidence for incomplete dominance of at least some loci conditioning this character.

SUMMARY

1. In order to obtain evidence of heritable variation in the carotene and crude protein content of orchardgrass, a selected group of plants from a combining ability experiment were analysed. The categories of material analysed included open-pollinated clones and fifth generation inbreds (I_5). The two classes of clones were crossed on two tester plants, one an unrelated open-pollinated clone of low carotene and protein content and the other an unrelated I_5 clone with a high analysis for the two constitueuts. In addition a limited number of single crosses between open-pollinated clones were examined.

2. Significant differences in content of carotene and crude protein were obtained for both the parents and the I_5 clones derived from them.

3. The test-cross data for open-pollinated clones showed a highly significant effect for testers with respect to carotene and significance at $P = 0.05$ for protein. Individual crosses between the open-pollinated clones and testers also differed significantly.

4. The mean tester effect in crosses using the I_5 clones lacked significance although slightly higher values were apparent for the high tester (426). The means of individual crosses with both testers showed highly significant differences for carotene and protein.

5. The correlation between carotene and crude protein was low in the open-pollinated clones and significant at $P = 0.05$ in the inbred (I_5) group. In three out of five groups of crosses the correlation between carotene and crude protein was significant and positive.

ACKNOWLEDGMENTS

The writer is indebted to H. R. Albrecht of the Department of Agronomy, the Pennsylvania State College, and to A. A. Hanson and V. G. Sprague of the U.S. Regional Pasture Research Laboratory, State College, Pennsylvania, under whose general direction this research was carried out. Appreciation is also expressed to H. R. Fortmann and H. L. Carnahan, Department of Agronomy, the Pennsylvania State College, and T. M. Stevenson, Forage Division, Central Experimental Farm, Ottawa, for helpful suggestions.

REFERENCES

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis 6th ed. Washington, D.C. 1945.
2. Aurand, L. W., R. C. Miller, and L. L. Huber. The influence of heredity on the carotene content of corn. Science 106 : 492–493. 1947.
3. Clarke, M. F. A study of the carotene and crude protein content of orchardgrass (*Dactylis glomerata*, L.). I. Variations due to stage of growth, cutting management and clones. Can. J. of Agr. Sci. 33 : 184-194. 1953.
4. Kohler, G. W., R. E. Lincoln, J. W. Porter, F. P. Zscheile, R. M. Caldwell, R. H. Harper and W. Silries. Selection and breeding for beta-carotene content (provitamin A) in tomatoes. Bot. Gaz. 109 : 219–225. 1947.
5. Millar, J. C., and H. M. Covington. Some factors affecting the carotene content of sweet potatoes. Am. Soc. Hort. Sci. 40 : 519–522. 1942.
6. Pickett, R. C. Variability of crude protein and carotene contents and their relations with other characters in bromegrass, *Bromus inermis* Leyss. Agron. J. 42 : 550–554. 1950.
7. Powers, Leroy. An expansion of Jones' theory for the explanation of heterosis. The Am. Nat. 78 : 275–280. 1944.
8. Tsiang, T. S. Variation and inheritance of certain characters of bromegrass (*Bromus inermis* Leyss). J. Am. Soc. Agron. 36 : 508–522. 1944.
9. Zscheile, F. P., and R. A. Whitmore. Determination of carotene in alfalfa. Ind. Eng. Chem., *Anal. Ed.* 19 : 170–172. 1947.

MOLYBDENUM POISONING OF CATTLE IN THE SWAN RIVER VALLEY OF MANITOBA[1]

HUGH M. CUNNINGHAM,[2] J. M. BROWN[3] and ARNOLD E. EDIE[4]

[Received for publication October 20, 1952]

ABSTRACT

Molybdenum poisoning of cattle caused by high concentrations of molybdenum in the forage has been found to exist in the Swan River Valley of Manitoba. Typical symptoms include severe diarrhoea, emaciation, anaemia, fading of hair colour, excessive craving for salt, and often death. Toxic herbage contained up to 25.6 p.p.m. of molybdenum while pasture samples from the Brandon and Winnipeg areas contained less than 1.9 p.p.m. The toxicity was overcome by the daily administration of 2 grams of copper sulphate as either a drench or salt lick. The disease was also produced experimentally in cattle by drenching with ammonium molybdate, but considerably more elemental molybdenum than found in toxic forage was required to induce the disorder in barn-fed cattle. Evidence was also obtained to show that the toxicity may pass through into the milk and affect the calf, but that cattle soon develop a resistance to the passage of ingested molybdenum into the milk.

INTRODUCTION

Molybdenum poisoning of cattle caused by high concentrations of molybdenum in the forage has been reported from various countries (1, 3, 5). The symptoms described by these workers have been found to closely resemble those displayed by cattle in a small area in the Swan River Valley of Manitoba. This disorder, which affects only cattle, has existed in this area for over 40 years. It has been observed to be particularly severe in cattle grazing on lush pasture or during seasons of heavy rainfall. When most severe, it may cause up to 30 per cent mortality in calves and even some death loss in mature stock.

The disease is marked by a persistent, severe, diarrhoea and an excessive craving for salt. The feces become exceedingly thin, bubbly, dark in colour, often containing large amounts of undigested matter. The diarrhoea is accompanied by a characteristic weakness of the legs and enlargement of the lower joints. The animals become stiff, show great difficulty in rising, and become greatly emaciated (Figure 1). As the disease progresses, affected animals develop a rough, staring coat, a gradual fading of hair colour, and a lowering of the haemoglobin content of the blood. Scouring stops abruptly if animals are moved to unaffected areas and begins again in 1 to 5 days if the animals are returned to the affected area.

EXPERIMENTS WITH COBALT

Cobalt deficiencies have been reported from various parts of Canada (6, 7, 8) and as the symptoms exhibited by the cattle in the Swan River Valley were quite similar to those of cobalt deficiency, an experiment was instituted on June 21, 1948, to determine if cobalt therapy would alleviate the condition. Sixty-three mgm. of elemental cobalt in the form of cobalt sulphate was administered weekly as a drench to 10 of 19 cattle pastured

[1] This work formed part of a thesis submitted by the senior author to the University of Manitoba, in partial fulfilment of the requirements for the degree of Master of Science.

[2] Department of Animal Husbandry, Cornell University Agricultural Experimental Station, Ithaca, N.Y.

[3] Head of Department of Animal Husbandry, the University of Manitoba, Winnipeg.

[4] Graduate Student, the University of Manitoba.

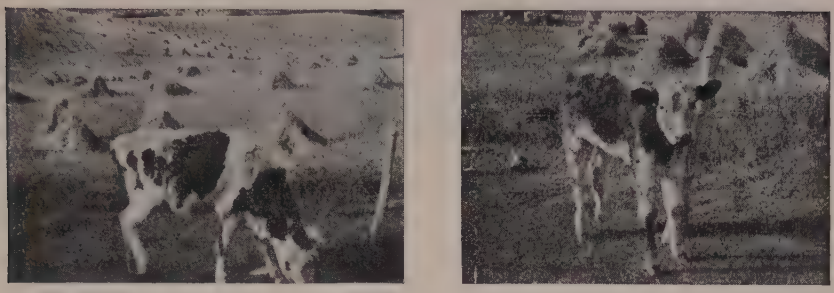


FIGURE 1. Calves affected with molybdenum poisoning showing unthriftiness and staring coat.

on four of the most severely affected pastures in the area. Weekly haemoglobin determinations were made on each animal using the Fisher Electro Hemometer. The experiment was run from June 21 to August 2. The scouring and other typical symptoms of the disorder were equally in evidence in both control and experimental groups and increased in severity with the advent of heavy rains. At no time during the seven weeks of the trial did the treated animals show any improvement over the controls in incidence of scours or in haemoglobin content of the blood.

EXPERIMENTS WITH COPPER SULPHATE

Noting the similarity in the symptoms of this disorder and those of molybdenum poisoning described by Ferguson and co-workers in England (5) it was considered advisable to employ their copper sulphate treatment for molybdenum poisoning. Accordingly, two grams of copper sulphate was administered as a daily drench to half the controls and to half the animals which had received cobalt in the previous experiment. Within two to three days the scouring subsided in the copper sulphate-treated group, the milk flow increased and the animals assumed a more thrifty and healthy appearance. There was no improvement in the untreated animals.

In search of a more practical method of administering copper sulphate, other than by drench, further experiments were carried out the following summer. As affected cattle exhibited an abnormal craving for salt, it was hoped that a salt lick containing copper sulphate would not only supply the corrective agent, but would provide an effective regulator in the therapy whereby stock would tend to consume more of the mix at a time when they most required it.

Based on the assumption that cattle in this region consumed an average of about 1 to 2 ounces of salt per animal per day, the following four levels of copper sulphate to salt were distributed among 13 herds in the affected area: 2 gram, 1 gram, $\frac{1}{2}$ gram and $\frac{1}{4}$ gram per ounce of salt. The copper sulphate-salt mix was fed ad libitum in wooden boxes and each herd was checked three times a week for incidence and degree of scouring, and salt-copper sulphate consumption. District rainfall and condition of pastures were also recorded.

The salt consumption was observed to increase considerably within one to two weeks after a series of heavy rains. It also increased when cattle were transferred from a sparse pasture to a lush pasture and then

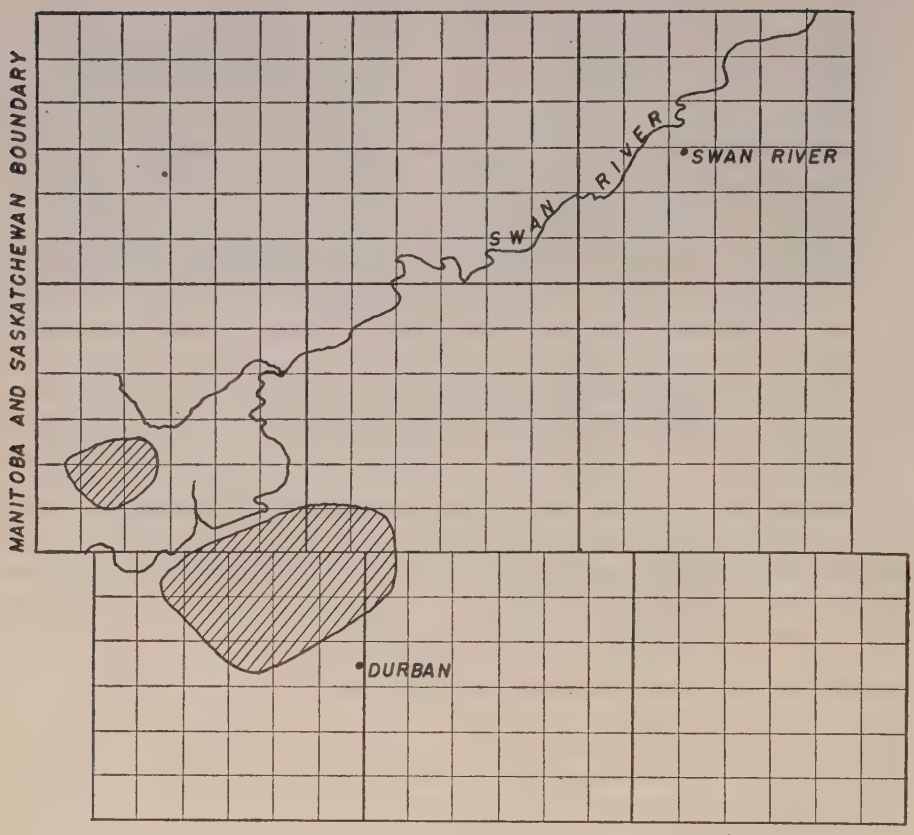


FIGURE 2. Map of nine townships in the western portion of the Swan River Valley showing the location of areas of high molybdenum toxicity (shaded sections).

decreased again as the pasture became poor. The higher levels of 2 and 1 grams of copper sulphate per ounce of salt were found adequate to prevent scouring throughout the season on the most severely affected parts of the area, while the lower levels of $\frac{1}{2}$ and $\frac{1}{4}$ grams were adequate on the outskirts of the area (Figure 2).

FORAGE ANALYSIS

During the summers of 1948 and 1949, forage samples were collected from the affected area and from widely scattered points about the province. These samples were analysed for molybdenum by the stannous chloride thiocyanide method (9). Duplicate determinations on dry weight basis were made on all samples.

Molybdenum was found in toxic quantities in toxic forage and its concentration was found to vary closely with the severity of the disorder. Nine out of 28 forage samples collected from affected pastures in the area contained over 10 p.p.m. of molybdenum [lower limit of toxicity set by American workers (4)] and two samples contained over 20 p.p.m. [Lower limit of toxicity set by the English workers (5)]. No samples secured from "safe" pastures either around the affected area or from other parts of the

province contained above normal concentrations of molybdenum. Six samples from pastures along the borders of the area varied from 1.0 to 6.4 p.p.m., while three samples from the Brandon and Winnipeg areas varied from 0.5 to 1.9 p.p.m. Oats and barley grain samples from the affected area did not contain toxic quantities of molybdenum, but still contained more than samples from the Brandon and Winnipeg areas. Barley from the affected area contained 2.9 and 5.6 p.p.m. and oats from the affected area showed 4.0 p.p.m. Barley from the Brandon area contained 0.7 p.p.m. and oats from the Winnipeg area, 1.2 p.p.m. of molybdenum.

Small concentrations of molybdenum in forage samples could usually be traced to "safe" pastures while toxic quantities of molybdenum were always associated with toxic pastures. Since affected pastures did not always produce scours after a period of drought or when the pasture was sparse, samples secured at such times similarly did not always contain over 10 p.p.m. of molybdenum. An excellent example of how the molybdenum content of the herbage varied with the severity of the disorder was a comparison between a "safe" and a highly toxic pasture lying just one mile apart. Grass from one pasture lying on low ground along the course of the Swan River which winds through the area had always been regarded as "safe" by farmers and contained only 1 p.p.m. of molybdenum. However, herbage from the most severely affected pasture in the area which lay on higher ground on the next farm contained 25.6 p.p.m. of molybdenum.

Unfortunately, equipment was not available for copper analysis of the forage to determine if a copper deficiency might be a contributing factor to the disorder. However, a copper deficiency does not appear to be the prime factor involved; animals introduced to toxic pastures become affected in a matter of days whereas if only a copper deficiency were involved, several months would be required for the development of the typical symptoms. Furthermore, much more copper sulphate is required to alleviate the disorder than should be required to treat a copper deficiency.

EXPERIMENTAL CAUSATION OF THE DISEASE

During the fall of 1948 experiments were conducted with cattle at the University of Manitoba to determine if molybdenum would produce the same syndrome as observed in affected cattle in the Swan River Valley. Five 2-year-old Holstein heifers constituted the experimental group. These were weighed before and after the experiment. The molybdenum dosage followed that used by Ferguson in England (5). Ammonium molybdate equivalent to 150 p.p.m. of molybdenum in 30 pounds of dry matter was administered as a daily drench to each animal. Within four days the heifers began to show the same symptoms as exhibited by affected animals in the Swan River Valley. At first there appeared a stiffness of legs, a slight looseness of feces, and a general listlessness. These symptoms became more intensified each day until the fourteenth day, at which time two animals were scouring so severely and the others were in such poor condition that it was necessary to discontinue the drenches. All animals but one lost weight during the 25-day period with an average loss of 44 pounds each.

ADDITIONAL EXPERIMENTS

During the winter of 1949-50 further experiments were conducted to determine if molybdenum could pass through the milk of affected cows in sufficient quantities to be toxic to calves. It had been noted in the field the previous summer that stall fed calves receiving no affected herbage, but receiving milk from cows grazing on toxic pastures scoured whenever the cows became affected and recovered upon recovery of the cows.

The experimental procedure followed was to administer molybdenum to a cow, analyse the milk for molybdenum, and observe any toxic effects on a calf receiving this milk. A daily drench of ammonium molybdate equivalent to 25 p.p.m. of molybdenum in 30 pounds of dry feed was administered to a 10-year-old cow and 10 pounds of her milk was given daily to a 4-months-old calf. The cow's roughage consisted of corn silage and alfalfa-brome hay. The milk was sampled at each milking and the samples composited for each 2-day period of the experimental period. The composite samples were then analysed in duplicate for molybdenum by a modification of the stannous-chloride thiocyanide method.

After 10 days of this treatment the cow failed to show any symptoms of molybdenum poisoning. The accompanying graph (Figure 3) also shows that there was no appreciable rise in the molybdenum content of the milk. The daily dose was then raised to an equivalent of 100 p.p.m. of molybdenum in 30 pounds of dry matter. During the following 23 days the molybdenum content of the milk gradually rose to over 2 p.p.m. and then dropped back to normal during the following 14 days. A further increase in the dosage to 200 p.p.m. similarly produced another rise and fall in the molybdenum content of the milk. Neither the cow nor the calf showed any symptoms of molybdenum poisoning.

As older animals have been found in the field to be more resistant than younger animals, it was considered advisable to experiment with a younger cow. Accordingly, a 5-year-old cow in her second lactation was given the

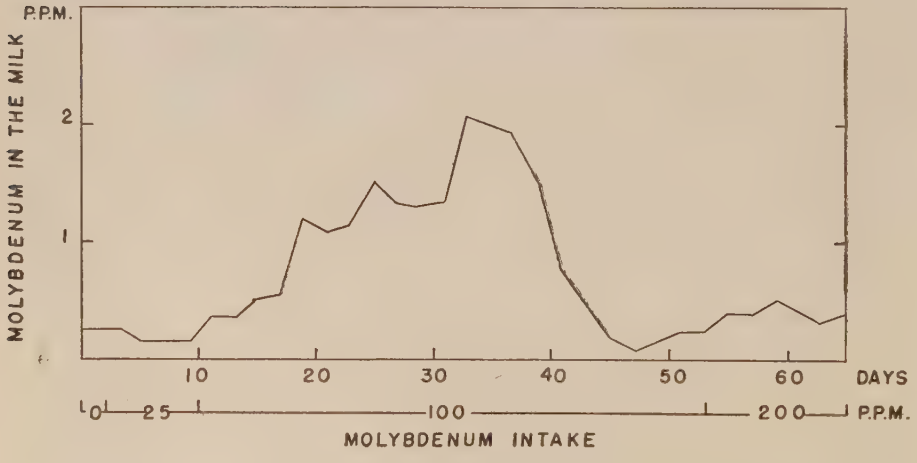


FIGURE 3. Graph showing the relation between the molybdenum content of the milk and the level of molybdenum intake. The daily molybdenum intake is expressed as parts per million in 30 pounds of dry matter.

100 p.p.m. molybdenum-intake level and 10 pounds of her milk was supplied daily to each of two 4-months-old calves. Haemoglobin determinations were made at frequent intervals on the cow and calves to determine if the molybdenum produced anaemia. After 20 days the dose was raised to 200 p.p.m. and after 7 more days to 400 p.p.m. At each level there was a subsequent rise and fall in the molybdenum content of the milk. Only when the dose was raised to an equivalent of 400 p.p.m. of molybdenum in 30 pounds of dry matter were toxic effects observed in the cow and calves. Scouring occurred in both the cow and calves, but other characteristic symptoms of molybdenum poisoning such as stiffness, unthriftiness, and staring coat were only observed in the calves and then only to a slight extent. The haemoglobin readings of the cow and calves which showed only slight reductions with previous doses reached the lowest levels when the animals began scouring (Table 1).

TABLE 1.—THE PASSAGE OF ORALLY ADMINISTERED MOLYBDENUM INTO THE MILK AND ITS EFFECT ON THE BLOOD HAEMOGLOBIN OF CALVES FED THIS MILK

Date	Daily molybdenum intake (p.p.m.)	Molybdenum in milk (p.p.m.)	Blood haemoglobin values (gm. per 100 ml.)		
			Cow	Calf No. 1	Calf No. 2
Jan. 1	100	0.23			
5	100	0.70	11.00	11.00	10.50
13	100	1.00			
19	100	0.98	9.00	9.50	10.25
21	200	0.95			
26	200	1.18	9.50	9.00	8.50
28	200	1.18			
30	200	1.18	10.50	9.00	8.00
Feb. 1	400	1.04			
2	400	2.33	10.50	9.00	8.00
4	400	2.73			
6	400	2.90	9.00	9.50	7.00

Analyses were also conducted on the milk of 10 other cows in the same herd to determine the normal molybdenum content of cows' milk on a typical ration in which the roughage consisted of corn silage and alfalfa-brome hay. The milk from these 10 cows varied from 0.03 to 0.35 p.p.m. of molybdenum with an average of 0.12 p.p.m. and a standard deviation of 0.10. These values compare closely with those obtained by Archibald (2) who, employing a spectrophotometric method, arrived at normal values for cows' milk of 0.018 to 0.147 p.p.m. with an average of 0.073 p.p.m. and Teresi (10) who reported an average of 0.045 p.p.m.

SUMMARY

Molybdenum poisoning of cattle caused by high concentrations of molybdenum in the forage has been shown to exist in a small area in the Swan River Valley of Manitoba. The disease has been corrected by the administration of copper sulphate in the form of a drench or salt-lick.

Cobalt was found to be of no benefit in alleviating the disorder. A copper deficiency in the forage may be a contributing factor, but the rapid onset of the disease and the large quantities of copper required to prevent it, exclude the possibility of a copper deficiency being the only factor involved.

The disease was experimentally produced in cattle by drenching with large doses of ammonium molybdate. Some evidence was also obtained to indicate that the toxicity may be transmitted through the milk to the calf. However, it was found that a cow is soon able to build up a resistance to the passage of ingested molybdenum through the milk.

REFERENCES

1. Allcroft, R. Nature (London) 158 : 796. 1946.
2. Archibald, J. G. Dairy Sci. 34 : 1026-1029. 1951.
3. Barshad, I. Soil Sci. 66 : 187-195. 1948.
4. Britton, J. W., and H. J. Goss. J. Amer. Vet. Med. Assoc. 108 : 176-178. 1946.
5. Ferguson, W. S., A. H. Lewis, and S. J. Watson. Bull. 1, Jealott's Hill Research Station, Imperial Chemical Industries Ltd., Bracknell, Berks. 1940.
6. Hamilton, R. Can. J. Comp. Med. 10 : 14. 1946.
7. Hanccock, E. E., and G. R. Smith. Can. J. Comp. Med. 9 : 312-313. 1945.
8. McIntosh, R. A. Can. J. Comp. Med. 9 : 179-182. 1945.
9. Piper, C. S. Soil and plant analysis. Univ. of Adelaide. 1942.
10. Teresi, J. D., C. A. Elvehjem, and E. B. Hart. Am. J. Physiol. 137 : 504-508. 1942.

THE RELATIONSHIP BETWEEN 30-, 60-, 90- AND 120-DAY NON-RETURNS TO SERVICE IN THE ARTIFICIAL INSEMINATION OF DAIRY CATTLE IN ONTARIO

T. D. Burgess[1]

Department of Animal Husbandry, Ontario Agricultural College, Guelph, Ont.

[Received for publication October 23, 1952]

ABSTRACT

The relationship between 30,- 60,- 90- and 120-day non-return to service in the artificial insemination of dairy cattle in Ontario as a measure of fertility has been studied. The data used for this study were drawn from the daily records of the Maple Cattle Breeders' Association, Maple, Ontario. In all 4,108 first services were considered. The per cent non-returns to service were calculated at 30, 60, 90 and 120 days after service. These percentages were then compared. It was found that the per cent non-returns decreased as the length of time after service increased up to and including 120 days. The differences found in per cent non-returns were in the following order: between 30- and 60-day non-returns, 8.7; between 60 and 90 days, 4.4 and between 90 and 120 days non-return 1.6 per centage units. The differences between 30 and 60 days non-return and between 60 and 90 days non-return proved to be significant. The difference between 90 and 120 days was not significant but approached significance.

INTRODUCTION

An artificial breeding unit must have some method by which relatively accurate estimates of fertility may be obtained. The only completely accurate method is of course the number of calves born in comparison with the number of services required to produce the calves. This, however, requires too much time before the results are known and a more rapid method is necessary. The use of pregnancy diagnosis is valuable but becomes impracticable where a large number of cows are involved.

The method most commonly used is based on the percentage non-returns to service at a given length of time after service. Cows not returning to service within a stated period of time are considered to be in calf. This method is the one used by Ontario Artificial Insemination Units. The most common period of time allowed for return to service is 60 to 90 days. Since some cows may not return to service until after the 90-day period has passed, it is evident, therefore, that non-returns to service as a measure of breeding efficiency is subject to error.

The error which is to be found in per cent non-returns to service is important when one is comparing per cent non-returns which have been calculated using different lengths of time for non-returns to service. In such a case it is necessary to adjust the percentages to a common basis. In order to do so the difference in non-returns at different intervals after service must be known.

The purpose of this investigation was to determine:

(1) The relationship between 30-, 60-, 90- and 120- day non-returns to service.

(2) The percentage of the cows showing delayed return to service.

[1] The data are taken from a thesis submitted by the author in partial fulfilment of the degree of Master of Science in Agriculture, 1952.

Salisbury (3) has reported a difference of 14.9 per cent in non-returns at one and five months after service. This difference was the same for both first and second services. Casida *et al.* (2) found that non-return to service calculations overestimated the level of conception as determined by pregnancy diagnosis at 35 to 49 days after insemination by the following amounts: 30 to 60 days after service, 15.3 per cent; 60 to 90 days after service, 6.1 per cent; 90 to 120 days after service, 3.0 per cent. Barrett *et al.* (1) in a similar work found the difference to be at 30 to 60 days, 14.9 per cent; 60 to 90 days, 5.5 per cent and at 90 to 120 days, 2.8 per cent.

MATERIAL AND METHODS

The data, for this study, were drawn from the daily records of the Maple Cattle Breeders' Association, Maple, Ontario, for the year 1947. These are a record of all the services performed each day throughout the year. These data were then subjected to statistical analysis using Fisher's analysis of variance methods. A total of 4,108 first services performed in the first nine months of that year were considered. Each service was checked for returns to service for a period of 120 days after service. The non-returns were totalled at 30, 60, 90 and 120 days after service. Percentage non-returns were calculated at each of these periods.

EXPERIMENTAL RESULTS

These data are presented in Table 1.

TABLE 1.—NON-RETURN TO SERVICE AT 30, 60, 90 AND 120 DAYS AFTER SERVICE FOR 4,108 SERVICES PERFORMED IN THE MAPLE UNITS IN 1947

Month	First services	30-day		60-day		90-day		120-day	
		N.R.	%	N.R.	%	N.R.	%	N.R.	%
January	426	361	84.7	318	74.6	306	71.8	299	70.2
February	363	307	84.6	270	74.4	258	71.1	247	68.0
March	330	277	83.9	257	77.9	246	74.5	239	72.4
April	510	416	81.6	365	71.6	326	63.9	319	62.5
May	435	364	83.7	321	73.8	312	71.7	306	70.3
June	773	586	75.8	520	67.3	495	64.0	478	61.8
July	548	441	80.5	394	71.9	368	67.1	366	66.8
August	345	269	78.0	247	71.6	227	65.8	222	64.3
September	378	295	78.0	262	69.3	246	65.1	244	64.5
Total	4108	3316	80.7	2954	71.9	2784	67.8	2720	66.2

An inspection of these data shows a decline in per cent non-returns as the length of time after service increases. There is a decline from 80.7 per cent at 30 days after service to 66.2 per cent at 120 days after service. When these data are subjected to statistical analyses the per cent non-returns prove to be significantly different in each case except the difference between 90- and 120-day non-returns which approaches significance.

DISCUSSION

From these data it is evident that per cent non-returns to service as a measure of breeding efficiency are subject to error. This error decreases as the length of time allowed to elapse after service increases before non-returns are taken. Therefore, if non-returns to service are to be used as a measure of breeding efficiency, an appreciable length of time must be allowed after service before non-returns are calculated in order to approximate actual pregnancies.

The mean difference in per cent non-returns at 30, 60, 90 and 120 days are in the following order: Between 30 and 60, 8.7; between 60 and 90, 4.2; and between 90 and 120, 1.6 percentage units. Therefore, when it is necessary to compare per cent non-returns calculated at different lengths of time after service, i.e. one at 60 days after service and one at 90 days after service, these per cent non-returns must be corrected in order to make a fair comparison. From these data 30-day non-return rates must be reduced by 8.7 ± 1.578 percentage units to compare with a 60-day non-return, by 12.9 ± 0.2161 percentage units to compare with 90-day non-returns, and by 14.4 ± 2.097 percentage to compare with 120-day non-return. A conception rate calculated on 60-day non-returns would have to be decreased by 4.2 ± 1.744 percentage units to compare with a 90-day non-return and by 5.7 ± 1.670 percentage units to compare with 120-day non-return. The 90-day non-return rate could be reduced by $1.6 \pm .8397$ percentage units to compare with a 120-day non-return rate.

The number of calves born as a result of the 4,108 first services studied is not known. Therefore the relationship between non-returns at 30, 60, 90 and 120 days and actual calvings cannot be determined. The work of Barrett and Casida (1) indicates that the 90- to 120-day non-returns overestimate pregnancies, as determined by pregnancy diagnosis at 35 to 49 days, by 2.8 to 3.0 per cent. It may be expected, therefore, that an additional 2 to 3 per cent of the cows will return to service at an interval greater than 120 days after service. In addition it is evident that some of the cows diagnosed as pregnant at 35 to 49 days may later abort. Due to these facts, neither non-returns to service nor pregnancy diagnosis can be considered as an accurate measure of the number of calves that will result from a given number of services. These data indicate, however, that the accuracy of non-returns to service increases as the period of time after service increases before non-returns to service are calculated.

Thirty days after service allows sufficient time for a cow to reach her next oestrus period if she did not conceive. According to Perry the interval between heat periods is 19 to 21 days. Therefore, a normal cow which did not conceive should have returned to service within the 30-day period allowed. In this study 4,108 cows were bred and 792 returned to service

within 30 days of the service date. However, at the end of 120 days after service 1,458 cows had returned to service. In other words 666 cows or 16.21 per cent of the cows inseminated returned to service at other than the normal interval.

The causes of a cow returning to service at a delayed interval may be many. It might be due to an early abortion, to some reproductive or hormonal abnormality in the cow or the owner may have "missed" seeing the cow in heat. What percentage of the delayed returns to service were due to specific causes is not brought out by these data. This involves further study.

SUMMARY

The relationship between 30-, 60-, 90- and 120-day non-returns for 4,108 inseminations has been studied. The mean difference between 30, 60, 90 and 120 days non-returns is in the following order: 8.7, 4.2, 1.6 percentage units, respectively. These results agree fairly closely with those of other writers mentioned in this paper. In comparing these results with those of Casida and Barrett (2) when pregnancy diagnosis was used, it would appear that approximately an additional 2 to 3 per cent of the cows will return to service at a time greater than 120 days after service. It is pointed out that non-return to service as a measure of breeding efficiency is useful for comparative purposes. It is not an accurate prediction as to the number of calves that will be born as a result of a given number of services. However, the accuracy of this measure of breeding efficiency increases as the length of time allowed to elapse after service increases.

Of the 4,108 cows bred 666 or 16.21 per cent returned to service at a longer interval after service than could be considered normal. The reasons for these delayed returns to service are not explained by the data at hand.

REFERENCES

1. Barrett, G. R., L. E. Casida, and C. A. Lloyd. Measuring breeding efficiency by pregnancy examinations and by non-returns. J. Dairy Sci. 31 : 682. 1948.
2. Casida, L. E., G. R. Barrett, and C. A. Lloyd.The use of pregnancy diagnosis with artificial breeding. J. Dairy Sci. 29 : 553. 1946.
3. Salisbury, G. W. The effect of time and other factors on the non-return to service estimate of fertility level in artificial insemination of cattle. J. Dairy Sci. 35 : 256. 1952.

WATER RETENTION AND AVAILABILITY IN SOILS RELATED TO DROUGHT RESISTANCE[1]

J. J. Lehane[2] and W. J. Staple[2]

Soil Research Laboratory, Swift Current, Sask.

[Received for publication October 16, 1952]

ABSTRACT

Observed differences in the available water-holding capacity of different textural classes of soil were not sufficient to account for the relative droughtiness of these soils. In most years the better soils in the Swift Current area, ranging from medium loams to heavy clays, could hold, within the depth of root penetration, all the moisture that reached the sub-soil.

Moisture release curves for different textural classes of soils were presented. It was suggested that the greater drought resistance of crops on the fine-textured clays might be related to the fact that the available moisture in these soils was held under greater stress than was the moisture in lighter soils. Other factors, such as soil temperature and aeration, should be considered in further experimental work.

A shortage of soil moisture reduces crop yields almost every year in the Brown and Dark Brown soil zones of the Prairie Provinces. It is a recognized fact that crops on heavy clay soils withstand drought better than those on lighter soils, and this has been generally attributed to the greater water-holding capacity of the clays. The exact nature of the drought resistance is not clear, however, because in many cases the crops on different textural classes of soil use almost equal amounts of water. In the present study the amount of available soil moisture held between the field capacity and the wilting percentage was determined for soils of different textures. In addition, the tension with which the moisture was held in these soils was measured. The percentage of available moisture when converted to inches of stored water shows which soils have sufficient storage capacity within the root zone to hold the pre-seasonal precipitation. Where storage is not a limiting factor, the relationship between available moisture and moisture tension, viz., the moisture release curve, may help to explain the greater efficiency of the finer-textured soils under semi-arid conditions. Field projects supply information on the moisture use and growth habits of crops produced on the same soils.

Certain limitations in the use of field capacity data should be mentioned. Veihmeyer and Hendrickson (11) have pointed out that the field capacity of soils is not an equilibrium value, but marks a slowing-down point in the drainage process. They describe the field capacity as the amount of water held in a soil after the excess water has drained away, and the rate of downward movement of water has materially decreased. This condition is usually reached within two or three days after a rain or irrigation in pervious soils of uniform structure and texture. Edlefsen and Bodman (3) and Hilgeman (5) have shown that the downward movement of moisture continues slowly for a long time after water is applied. This rate of movement is so slow, compared to the use of water by plants, that for practical irrigation purposes the field capacity is a satisfactory measure of the

[1] Contribution from the Division of Field Husbandry, Soils and Agricultural Engineering, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Canada.

[2] Agricultural Research Officer, Soil Research Laboratory, Swift Current, Saskatchewan.

amount of water a soil will hold under a growing crop without loss below the root zone. On the other hand, if the top 4 feet or normal root depth in a fallowed soil is irrigated to field capacity, considerable moisture is likely to be lost by subsequent drainage. Hilgeman (5) showed that a silty loam soil lost 3 inches of water by drainage from the top 4.5 feet of soil from the fifth to the sixtieth day after irrigation.

Various workers have shown that the forces, with which water is held by the soil, influence plant growth and drought resistance. Wadleigh *et al.* (12, 13, 14) found that the growth of bean and guayule plants was controlled by the total moisture stress in the plants, whether produced by moisture tension in a dry soil or by osmotic pressure in a saline soil. They showed also that leaf elongation of cotton plants decreased as a second degree function of the soil moisture stress. Furr and Reeve (4) observed that soils had a wilting range that generally increased with increased fineness of texture. The tension at the upper end of this range averaged 9 atmospheres and at the lower end 22.4 atmospheres. They showed that at the upper end of the range plant growth ceased, and soil moisture within the wilting range provided the plant with an emergency reservoir that would likely enable many species of plants to survive periods of prolonged drought, or to mature seed after vegetative growth had ceased. Staple and Lehane (9) reported that under dry conditions plants used some moisture below the wilting point that would likely be beneficial to the grain yield. They also observed, when growing plants under moisture stress in sealed gallon crocks in the greenhouse, that crops on fine-textured soils yielded higher for the amount of water used than those on coarse-textured soils.

METHODS

Laboratory analyses were made on surface soil samples, representing a wide range in texture, collected at Experimental Substations in southwestern Saskatchewan. All soils were passed through a 2-mm. sieve before determinations were made. The coarse and very fine sand were obtained by washing and screening. The sands were included in the analysis because many of the sandy soils in this area are underlain with almost 100 per cent sand.

For the field capacity determinations, air-dried soils were packed uniformly in cylinders 2 inches in diameter and 10 inches high. Sufficient water was added to wet the soil after prolonged drainage to a depth of 8 to 9 inches, and the top was sealed to prevent evaporation. After 72 hours the upper 2 inches of soil was removed, and a sample was taken from the next 2 inches for moisture determination. The field capacity was expressed as a percentage of the oven-dry weight of the soil. Permanent wilting percentages were determined by the direct method of growing dwarf sunflowers as outlined by Work and Lewis (15). The field capacity minus the wilting percentage gave the available moisture capacity or the percentage of moisture that was available for crop growth. Moisture equivalents were determined by the standard centrifuge procedure of Briggs and Shantz (2). Mechanical analyses were made by the revised Bouyoucos hydrometer method (1).

Apparent specific gravity or volume weight was determined by weighing cores of undisturbed soil, 2 inches in diameter and 2 inches high, taken

TABLE 1.—MOISTURE CONSTANTS AND AVAILABLE MOISTURE CAPACITY OF SOILS

Soil association and textural class	Mechanical analysis			Moisture equivalent	Field capacity	Permanent wilting percentage	Apparent specific gravity	Depth of root penetration	Available moisture in root zone	
	Sand	Silt	Clay						At field capacity	From normal storage capacity at seeding time
	%	%	%	%	%	%		ft.	in.	in.
Coarse sand	100	—	—	1.14	3.00	0.78	1.70	5.0	2.2	1.1*
Very fine sand	100	—	—	6.54	10.20	3.42	1.60	5.0	6.5	3.9*
Hatton very fine sandy loam	71	21	8	12.78	15.95	5.77	1.55	4.5	8.5	5.0
Wood Mountain clay loam	32	46	22	21.40	22.80	8.77	1.36	4.0	9.1	6.0
Fox Valley silty loam	30	52	18	21.80	23.20	9.13	1.26	4.0	8.5	5.7
For Valley silty clay loam	26	53	21	24.00	24.26	9.82	1.26	4.0	8.7	6.0
Sceptre clay	21	47	32	28.69	27.30	13.14	1.30	4.0	8.9	6.5
Fox Valley silty clay	5	54	41	31.25	30.95	14.35	1.28	3.7	9.5	6.7
Sceptre heavy clay	10	30	60	43.00	40.82	21.35	1.20	3.5	9.8	7.1

* Moisture 4 months after water added in cylinders

at different depths in the field. The product 0.01 X available moisture percentage X apparent specific gravity gave the amount of water (inch) stored per inch depth in the soil profile. In field sampling, 5 samples were taken for moisture determination to a depth of 4 feet in sections of 0-6, 6-12, 12-24, 24-36 and 36-48 inches. The water storage in inches was calculated separately for each section, the final sum giving the amount available in the 4-foot depth.

The depth of root penetration was the average depth to which cereal crops removed moisture by harvest time in years of ample stored moisture. The moisture release curves were obtained for tensions up to one-half atmosphere with the tensiometer, and for 1 to 15 atmospheres with Richards' (6) pressure membrane apparatus.

RESULTS AND DISCUSSION

Water Holding Capacity

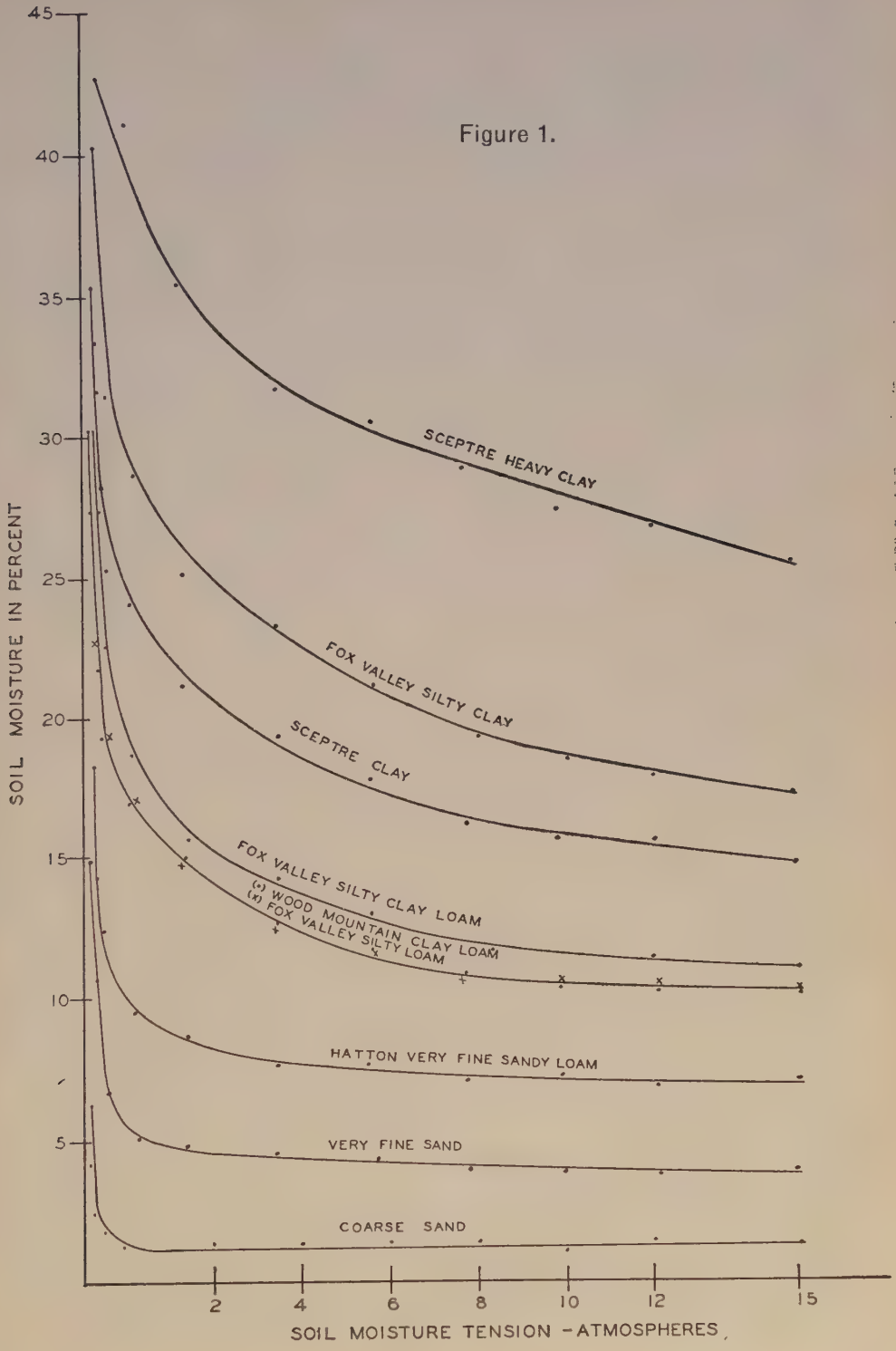
Physical analyses and moisture data for the soils are presented in Table 1. The range in texture was from 100 per cent sand to 60 per cent clay. The moisture equivalent and field capacity were nearly equal for the medium-textured soils, but the former was lower for the sands and sandy loams. The depths of root penetration apply, of course, only in years of ample precipitation during the summerfallow period. In dry years, the stored moisture is insufficient to moisten the soil to these depths.

The data in the second-last and last columns of Table 1 might be termed respectively the "theoretical" and "actual" moisture available to crops under dry-land farming when the soil is moistened throughout the root zone. The second-last column shows the amount of water in inches held between the wilting point and the field capacity. The soil could hold this maximum for only a short time. The last column, obtained from field sampling (8), shows the average moisture held at seeding time when the soil was moistened to the depth of root penetration. This occurred only in the wettest years of the 14-year experimental period. The variation in the data was high, about $\pm$ 1.5 inches, because of differences in rainfall distribution and subsequent moisture movement. The data for the two sands were obtained by permitting sealed 4-foot cylinders of sand, with the upper half initially at or above field capacity, to drain for four months. This treatment for sandy soils yielded a final moisture content near that found under field conditions at seeding time.

Table 1 shows that the moisture actually available to the crop was 27 to 50 per cent less than the maximum based on field capacity. This was in general agreement with the observations of Edlefson and Bodman (3) and of Hilgeman (5), mentioned previously. Presumably the water in the larger pore spaces was redistributed by capillary conductivity, part of it moving upward to evaporate at the surface, and part downward into drier soil near the bottom of the root zone. Under average conditions, the loss at the surface is reduced by the loose, dry surface soil, and is offset at intervals by additional rainfall. The downward movement is readily observed in successive samplings, even when little or no effective rainfall is received.

Under dry-land farming in southern Saskatchewan the fields are usually summerfallowed every second year to conserve moisture. The important

Figure 1.


45
40
35
30
25
20
15
10
5
SOIL MOISTURE IN PERCENT
SCEPTRE HEAVY CLAY
FOX VALLEY SILTY CLAY
SCEPTRE CLAY
FOX VALLEY SILTY CLAY LOAM
(•) WOOD MOUNTAIN CLAY LOAM
(×) FOX VALLEY SILTY LOAM
HATTON VERY FINE SANDY LOAM
VERY FINE SAND
COARSE SAND
2
4
6
8
10
12
15
SOIL MOISTURE TENSION - ATMOSPHERES

factor is the amount of available water the soil will store for crop use in the 21-month summerfallow period. When most of the moisture is received early in the period the subsequent penetration is deeper, and the percentage moisture at any depth is less, than when received during the second fall and winter. Stubble fields which are dry in the fall, and hold deep snow-drifts over winter, usually contain more water per foot of moist soil at seeding time than do summerfallow fields.

Sandy soils differ greatly in their capacity to hold water. The available moisture-holding capacity of a sandy soil underlain with coarse sand may be only a few inches, whereas that of a sandy soil with subsoil of a loamy texture or very fine sand may be as high as 5 to 6 inches. The mean moisture conservation in the summerfallow period in southern Saskatchewan is 4 inches (8), and under average weather conditions each additional inch of stored water will increase the yield by 3 to 5 bushels per acre (7). It appears, then, that the moisture-holding capacity of the coarser sandy soils is a serious limiting factor in crop growth. On the other hand, the sandy soils with permanent wilting percentages of 5 per cent or over will hold, within the depth of root penetration, 4 inches of water or the average found in summerfallowed fields. When above-average precipitation is received some moisture may be lost below this depth. The loams, clay loams and clays will lose moisture below the root zone only in the wettest years (about three out of ten). Apparently the direct effects of differences in water-holding capacity do not alone account for the relative droughtiness of different soils.

Soil Moisture Stress

Figure 1 gives the moisture release curves of the seven soils and two sands studied, over the entire tension range that will permit the growth of crops. The soil moisture content in per cent as ordinate is plotted against the moisture tension in atmospheres as abscissa. The field capacity corresponds to the moisture content at about $\frac{1}{3}$ atmosphere tension, at least for medium- and fine-textured soils, and the wilting percentage corresponds to that at somewhat greater than 15 atmospheres.

A significant feature of the series of curves is that they tend to straighten out, and cut the coordinate axes at a greater angle in going from sands to clays. The sands hold most of their available moisture under very low tensions, whereas with increasing clay content, the soils hold proportionately less available moisture under low tensions and more under high tensions. As various authors have pointed out, the moisture release curves explain why, under field conditions, all the moisture held by the lighter soils between field capacity and wilting point may appear to be equally available to crops.

Table 2 gives the available moisture held in a 1-foot depth of soil at different moisture stresses. These data were obtained from Figure 1, subtracting the wilting percentage from the soil moisture held at different stresses, and multiplying by 0.12 X apparent specific gravity to give the available moisture in inches. Columns 2 and 3 of Table 2 were obtained by dividing the last two columns of Table 1 by the depth of root penetration. Comparing the normal storage data in column 3 with the moisture held at

TABLE 2.—THE AVAILABLE MOISTURE HELD IN A 1-FOOT DEPTH OF SOIL AT DIFFERENT MOISTURE TENSIONS

Soil association and textural class	Available moisture										
	Field capacity	Normal storage at seeding time	$\frac{1}{3}$ atmos.	1 atmos.	2 atmos.	4 atmos.	6 atmos.	8 atmos.	10 atmos.	12 atmos.	15 atmos.
	in.	in.	in.	in.	in.	in.	in.	in.	in.	in.	in.
Coarse sand	0.44	0.22	0.06	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Very fine sand	1.30	0.78	0.60	0.29	0.21	0.11	0.09	0.04	0.02	0.02	0.02
Hatton very fine sandy loam	1.88	1.11	0.93	0.68	0.49	0.31	0.28	0.20	0.19	0.16	0.16
Wood Mountain clay loam	2.28	1.50	1.61	1.30	0.98	0.59	0.42	0.24	0.21	0.19	0.16
Fox Valley silty loam	2.12	1.43	1.53	1.21	0.80	0.48	0.32	0.20	0.15	0.15	0.10
Fox Valley silty clay loam	2.18	1.50	2.15	1.23	0.85	0.60	0.44	0.26	0.23	0.20	0.14
Sceptre clay	2.22	1.62	2.18	1.61	1.20	0.94	0.69	0.44	0.36	0.33	0.22
Fox Valley silty clay	2.56	1.87	2.82	2.09	1.58	1.30	1.00	0.75	0.65	0.51	0.41
Sceptre heavy clay	2.80	2.03	2.94	2.19	1.97	1.50	1.30	1.06	0.83	0.61	0.55

$\frac{1}{3}$ and 1 atmosphere tensions, it appears that much of the loosely-held water was already either lost or redistributed by capillarity at seeding time. In general, the percentage reduction due to moisture movement is less in the clays than in the lighter soils because the former hold less of the available water in the low tension range.

The force which plant roots must exert to remove the available moisture from different soils is emphasized by comparing the mean moisture at seeding time with the moisture held at 8 atmospheres. About 50 per cent of the moisture available to the crop on Sceptre heavy clay is held with a stress greater than 8 atmospheres, whereas only 16 per cent of the moisture in Wood Mountain clay loam is held with such a stress.

A plausible theoretical explanation, in terms of the moisture-stress relationship, might be proposed to account for the greater drought resistance of heavy clay soils. In a dry year the crops that make a slow growth in early June, and produce a minimum of leafage, are able to save the stored moisture until it is most needed for heading and filling in July. It seems possible that the greater stress on the available moisture in clays may contribute to such a result in modifying the moisture use and crop growth on these soils early in the season. Crops on sandy soils make a lush early growth and stool heavily, using up most of the available moisture under a minimum of stress. By the time moisture tension begins to affect the absorption by plant roots, the supply of available water is almost exhausted. Unfortunately definite experimental evidence on the role of moisture stress in the over-all efficiency of moisture use is limited. Wadleigh and Gauch (14) showed that the rate of leaf growth depends on the soil moisture stress. Staple and Lehane (9) showed that crops grown under moisture stress on clay soils in crocks were more efficient than those on loams. At the minimum moisture level the crops on clay outyielded the best crops on loam by about 20 per cent, whereas at the high moisture level the loams outyielded the clay.

Soil moisture and yield data collected on Experimental Substations since 1937 supply additional information on this problem. The results obtained thus far are summarized in the following regression equations relating wheat yield in bushels per acre (Y), seasonal rainfall in inches (R), and stored moisture used by the crop in inches (S):

$$\text{Loams (131 station-years)} \quad Y = 3.4R + 2.8S - 15$$
$$\text{Clays (30 station-years)} \quad Y = 2.5R + 4.3S - 11$$

Some changes may be necessary in these equations when more data become available. For the loams, the increase in yield for each additional inch of water used is greater for rainfall than for stored water, but for the clays the reverse is true. The relative yields predicted for clay and loam are in general agreement with field observations made in districts where such soils are adjacent. When the seasonal precipitation and stored moisture used are near average, 6 and 4 inches, respectively, the predicted yields are 20 bushels per acre for clay and 16 bushels per acre for loam. With low precipitation and average storage, i.e., with $R = S = 4$ inches, the yields are 15 bushels per acre for clay and 9 bushels per acre for loam. At much higher rainfalls the relative efficiency of the soils is reversed, and often crops on loam soils outyield those on clay. Presumably the wheat plant

removes the stored moisture (S) under a greater average stress than it does the portion of the rainfall (R) which it transpires, and the high regression of yield on stored moisture for the clays supports the belief that the stress on this moisture is a controlling factor in the efficiency of moisture use.

Under field conditions there are undoubtedly a number of other factors, such as soil temperature and aeration, which influence the relative efficiency of crops on soils of different texture. Also, the mean stress at the roots of a growing crop in the field is difficult to establish because it is controlled not only by the moisture release curve of the soil, but by the distribution of moisture in the soil profile, the root penetration (also influenced by soil texture), and weather conditions such as rainfall, humidity, temperature, etc., which influence the rate of transpiration. A method is needed for integrating these factors into a single index of stress or relative availability of soil moisture with which growth and ultimate yield can be correlated. The recent work by Taylor (10) is a promising step in this direction.

REFERENCES

1. Bouyoucos, G. J. A recalibration of the hydrometer method for making mechanical analysis of soils. Agron. J. 43 : 434-438. 1951.
2. Briggs, L. J., and H. L. Shantz. The wilting coefficient for different plants and its indirect determination. U.S.D.A. Bur. Plant Indus. Bull. 230 : 10. 1912.
3. Edlefsen, N. E., and G. B. Bodman. Field measurement of water movement through a silty loam soil. J. Amer. Soc. Agron. 33 : 713-731. 1941.
4. Furr, J. R., and J. O. Reeve. Range of soil moisture percentage through which plants undergo permanent wilting in some soils from semi-arid irrigated areas. J. Agr. Res. 71 : 149-170. 1945.
5. Hilgeman, R. H. Changes in soil moisture in the top eight feet of bare soil during 22 months after wetting. J. Amer. Soc. Agron. 40 : 919-925. 1948.
6. Richards, L. A. Pressure membrane apparatus construction and use. Agr. Eng. 28 : 451-454. 1947.
7. Soil Research Laboratory. Soil moisture, wind erosion and fertility of some Canadian prairie soils. Canada Dept. of Agr. Pub. 819. 1949.
8. Staple, W. J., and J. J. Lehane. The conservation of soil moisture in southern Saskatchewan. Sci. Agr. 32 : 36-47. 1952.
9. Staple, W. J., and J. J. Lehane. The use of the wilting coefficient in soil moisture studies in southwestern Saskatchewan. Sci. Agr. 21 : 440-447. 1941.
10. Taylor, S. A. Estimating the integrated soil moisture tension in the root zone of growing crops. Soil Sci. 73 : 331-339. 1952.
11. Veihmeyer, F. J., and A. H. Hendrickson. Methods of measuring field capacity and permanent wilting percentages of soils. Soil Sci. 68 : 75-94. 1949.
12. Wadleigh, C. H., and A. D. Ayers. Growth and biochemical composition of bean plants as conditioned by soil moisture tension and salt concentration. Plant Physiol. 20 : 106-132. 1945.
13. Wadleigh, C. H., H. G. Gauch, and O. C. Magistad. Growth and rubber accumulation in guayule as conditioned by soil salinity and irrigation regime. U.S. Dept. of Agr. Tech. Bull 925 : 1-34. 1946.
14. Wadleigh, C. H., and H. G. Gauch. Rate of leaf elongation as affected by the intensity of the total soil moisture stress. Plant Physiol. 23 : 485-495. 1948.
15. Work, R. A., and M. R. Lewis. Moisture equivalent, field capacity and permanent wilting percentage and their ratio in heavy soils. Agr. Eng. 15 : 355-362. 1934.

STUDIES ON THE EFFECT OF LIGHT ON THE GROWTH AND DEVELOPMENT OF RED CLOVER[1]

R. A. Ludwig[2], H. G. Barrales[3] and H. Steppler[4]

[Received for publication October 27, 1952]

ABSTRACT

The effect of light, particularly photoperiod, on the growth and development of the Dollard variety of red clover was studied.

Inherent morphological variability showed maximum expression under conditions of short daylength and minimum expression under long day conditions. Exposure to high light intensity during the period of illumination tended to mask the effect of short daylength.

Individual plant lines were found to vary in their ability to survive under conditions of low light intensity. This indicates that strains of red clover, more adapted for growth with a nurse crop, could be developed by breeding.

A 14-hour photoperiod was found to be critical for flowering in most of the clonal lines studied. Some lines, however, required a 16-hour photoperiod.

Flowering was more profuse on plants exposed to 16-hour photoperiods than it was on those exposed to 14-hour photoperiods on all but two of the lines studied. In the latter case the reverse was true.

The duration of photoperiodic stimulation required to induce flowering was different with different plants. Inadequate length of stimulation often resulted in the production of abortive inflorescences.

INTRODUCTION

Red Clover (*Trifolium pratense*) is one of the most important forage legumes grown in Eastern Canada. The strains now commonly grown have been selected largely on the basis of winter survival and yield. As pointed out by Bird (2), not only do differences occur between strains but even larger differences often occur between the individual plants comprising a single strain. Among the more conspicuous of these differences are the morphological ones, but physiological ones obviously also occur and result in differences in such properties as disease resistance and winter hardiness. The studies reported in this paper deal with the effect of light, particularly photoperiod, on the growth habit and development of red clover. They were undertaken to determine to what extent observed morphological differences represent differences in physiological response to environment.

MATERIALS AND METHODS

The Dollard variety of red clover was employed in the studies reported in this paper. The plants used in the first experiment were grown from seed but in all subsequent experiments they were propagated vegetatively from individual plant lines, thus permitting an accurate comparison between treatments. Most of the clonal lines used were obtained from among the plants grown in the first experiment and were selected for apparent diversity of type rather than for any agronomic characteristic. A few of the clonal lines originated from field-selected plants.

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Quebec. Journal Series No. 312.

[2] Principal Plant Pathologist, Science Service Laboratory, University Sub Post Office, London, Ontario. Formerly Associate Professor of Plant Pathology, Macdonald College (McGill University), Quebec.

[3] Graduate Assistant, Department of Botany, University of Toronto. Formerly Graduate Assistant, Department of Agronomy, Macdonald College (McGill University), Quebec

[4] Associate Professor of Agronomy, Macdonald College (McGill University), Quebec.

Vegetative propagation of the experimental plants was accomplished by means of crown cuttings taken from plants grown under conditions of eight-hour daylength. The technique employed in taking and handling the cuttings has been described elsewhere (1).

Daylength was regulated by either covering the plants to shorten the normal daylight period or illuminating with incandescent lamps to extend the normal daylight period. Light-tight frames covered with heavy building paper were used for darkening. When artificial illumination was necessary an attempt was made to maintain a minimum intensity of 100-foot candles at the plant level. Unless otherwise stated in the text, the term "short day" refers to a light exposure period of 8 hours per day, while the term "long day" refers to a light exposure period of 16 hours per day.

THE EFFECT OF SHORT AND LONG DAYS ON THE GROWTH AND DEVELOPMENT OF RED CLOVER PLANTS PROPAGATED FROM SEED

The effect of length of day on the growth habit of red clover plants grown from seed was studied in an initial experiment. In this experiment plants were grown in 6-inch flower pots in the greenhouse. A number of seeds of Dollard red clover were planted in each pot and after emergence the seedlings were reduced to one per pot. Throughout the experiment the day temperature was maintained as close to 70° F. and the night temperature as close to 55° F. as possible. "Day length" treatments were begun as soon as the first leaf was well developed. The treatments used consisted of the exposure of plants to either continuous short-day conditions, continuous long-day conditions or to different combinations of short and long days. Exposure periods of one month's duration were employed and the experiment continued for four months. All combinations of short and long days were used. Thus, treatment No. 1, of the resultant 16 treatments, consisted of continuous exposure to short days; treatment No. 2 of exposure to short days for the first three months and long days for the fourth month; and treatment No. 16 of exposure to continuous long-days. Each treatment consisted of three plants and was replicated four times, giving a total of 12 plants per treatment. The treatments were placed at random within replicates.

The plants under long days made much more growth during the first month than did those under short days. This was apparent in the greater petiole elongation and leaflet expansion occurring under long-day conditions. The short-day plants were approximately $1\frac{1}{2}$ to 2 inches in height at this time as compared to 4 to 6 inches in the long-day plants. Development within treatments was very uniform.

The differences noted at the end of the first month persisted throughout the second month in those plants that continued under the original long- or short-day conditions. Plants that were under short-day conditions for the first month and were then transferred to long-day conditions for the second month developed as if they had been under continuous long days, i.e., long petioles, large leaflets and upright growth habit. The plants within this treatment behaved uniformly. Plants that were under long days for the first month and short days for the second month developed a prostrate habit of growth similar to those exposed to continuous short-day

conditions. Unlike the plants in other treatments, those receiving this treatment showed considerable variability. This was apparent in leafiness, crown bud development, and openness of crown.

The growth habit and general appearance of the plants at the end of the third month is illustrated in Plate I. It will be noted, first of all, that the plants receiving long days during the third month are erect in their growth habit while those receiving short days are prostrate. Continuous exposure to long days induced flowering early in the third month under the conditions of the experiment. Flower induction also occurred in plants that had received the long-day treatment during the first two months and the short-day treatment during the third month, but the appearance of flowers was delayed as compared to those receiving continuous long days. A single month of exposure to long days was not adequate to induce flowering if it occurred in either the first or second month of development, but was when applied in the third month. A month of short days interposed between two months of long days resulted in best vegetative growth. Flower production was delayed but not inhibited.

During the fourth month of the experiment all plants exposed to long days came into bloom if they had not already done so under previous treatments. Plants exposed to continuous short days or receiving only one month of long days in either the first or second month failed to bloom. Exposure to long days in the fourth month following short days in the first three months was adequate to induce flowering.

THE EFFECT OF SHORT AND LONG DAYS ON THE GROWTH AND DEVELOPMENT OF VEGETATIVELY-PROPAGATED RED CLOVER PLANTS

Twenty-four clones from each of six clonal lines were used in this experiment. Half of the plants, 12 from each clonal line, were held under "short-day" conditions for one month after taking the cuttings, before commencing the treatments. The other half were held for two months. This was done in order to determine the effect of age of plant on photoperiodic stimulation. The treatments consisted of the exposure of two plants per clone from each of the above series to continuous short days, two to each of 3-, 7-, 15- and 21-day periods of long days followed by return to short-day conditions, and two to continuous long days.

The flowering date and the number of flowers formed were recorded for each clonal line in the different treatments.

With certain treatments some of the clonal lines failed to flower but produced abortive inflorescences. In these inflorescences the development of the florets was completely arrested on the body of the receptable. The treatments inducing the formation of normal, fully developed, inflorescences and the number of days required for this are given in Table 1.

It is obvious from these data that the age of the plant greatly influences its sensitivity to photoperiodic stimulation. Flowering was only induced by 15- and 21-day periods of exposure to long days in plants that were two months of age at the time the treatment was commenced. If continuously maintained under long days, however, both one- and two-month-old plants flowered. Increasing the length of exposure to long days and increasing

PLATE I. Growth and development of 3-months-old red clover plants grown from seed under varying conditions of daylength.

A. Continuous short day.
B. Short days first two months, long days third month.
C. Short days first month, long days last two months.
D. Short days first and third months, long days second month.
E. Long days first month, short days last two months.
F. Long days first and third months, short days second month.
G. Long days first two months, short days third month.
H. Continuous long day.

PLATE II. The effect of length of photoperiod on the growth and development of four typical red clover clones

Top to bottom—clonal lines
Left to right—8-, 10-, 12-, 14- and 16-hour photoperiods.

TABLE 1.—THE EFFECT OF PLANT AGE AND LENGTH OF EXPOSURE TO LONG DAYS ON THE NUMBER OF DAYS REQUIRED FOR THE PRODUCTION OF FULLY DEVELOPED INFLORESCENCES

Clonal line number	Days exposure to 16-hour photoperiods											
	One-month-old plants						Two-months-old plants					
	0	3	7	15	21	Continuous	0	3	7	15	21	Continuous
159	—*	—	—	—	—	43	—	—	—	67	57	41
137	—	—	—	—	—	43	—	—	—	67	57	40
146	—	—	—	—	—	47	—	—	—	57	44	41
147	—	—	—	—	—	49	—	—	—	—	65	46
102	—	—	—	—	—	52	—	—	—	—	63	Dead
112	—	—	—	—	—	59	—	—	—	—	—	52

* Indicates failure to flower.

TABLE 2.—THE EFFECT OF PLANT AGE AND LENGTH OF EXPOSURE TO LONG DAYS ON THE NUMBER OF FULLY DEVELOPED AND ABORTED INFLORESCENCES PRODUCED

Clonal line number	Days exposure to 16-hour photoperiods								
	One-month-old plants			Two-months-old plants					
	15 aborted	21 aborted	Continuous normal	7 aborted	15		21		Continuous normal
					Normal	aborted	Normal	aborted	
159	—	1	2	2	1	3	2	—	4
137	3	5	8	2	1	11	2	12	9
146	—	1	7	1	2	—	5	—	6
147	—	3	4	—	—	3	1	1	3
102	—	—	5	—	—	6	1	2	Dead
112	—	1	4	—	—	1	—	—	3

the age of the plant reduced the number of days required for flowering. "Long-day" exposure periods of less than 15 days were ineffective in inducing the formation of fully developed inflorescences.

Table 2 gives the effect of the length of the "long-day" exposure period on the number of fully developed and aborted inflorescences produced in each of the clonal lines.

Inflorescence formation was completely inhibited in treatments other than those indicated in this table. The number of inflorescences produced by one- and two-months-old plants do not differ significantly when they are grown under conditions of continuous exposure to "long days".

Under exposure conditions of 21 days or less, however, a greater number of inflorescences are produced by the older plants. Further, it is obvious from the data presented that the "long days" are not only required for the differentiation of the floral primordia but also for the further development of inflorescences. Finally, the data presented in Tables 1 and 2 clearly show a difference in the response of individual plant lines to daylength treatment.

THE EFFECT OF VARYING DAYLENGTH ON GROWTH AND DEVELOPMENT

Only two lengths of day were employed in the experiments reported in the preceding sections. These were short days (8 hours) and long days

(16 hours). Since they represent two extremes, and do not provide information on either the minimum daylength required for flowering or the effect of intermediate daylengths on growth and development, an experiment was undertaken in which the effects of series of daylengths were studied.

Fourteen clonal lines of red clover were used in this experiment. These were propagated by means of crown cuttings in the usual manner and held under conditions of 8-hour daylength for approximately four weeks prior to the initiation of the treatments. Five treatments consisting respectively of continuous exposure to 8-, 10-, 12-, 14- and 16-hour photoperiods were employed. Three plants from each clonal line were placed under each treatment. The temperature of the greenhouse was maintained between 65° F. and 75° F. during the course of the experiment.

The heights of the plants were measured after one month's treatment and again after four months' treatment. At the same time, counts were made of the number of leaves formed on each plant. The data obtained are presented in Table 3 as an average for all clones.

It can be seen from these data that the plant height had increased and the numbers of leaves decreased very regularly with increasing length of photoperiod during the first month's exposure. The data recorded after four months of growth show the stimulating effect of long daylengths and the lack of stimulation of short daylengths on increase in plant height. It results from the promotion of stem elongation in the former and its inhibition in the latter. The number of leaves produced during four months increased with decreasing daylength down to photoperiods of 10 hours. The fact that this increase did not extend to the eight-hour photoperiod might be explained on the basis of impaired photosynthetic activity.

TABLE 3.—AVERAGE HEIGHT AND NUMBER OF LEAVES PER PLANT UNDER DIFFERENT PHOTOPERIODS

Length of photoperiod	Average plant height (cm.)		Average number of leaves per plant	
	After 1 month's treatment	After 4 months' treatment	After 1 month's treatment	After 4 months' treatment
8	3.44	5.13	16.03	113.16
10	5.01	8.50	13.58	122.50
12	6.67	12.10	13.19	98.63
14	10.75	24.74	11.26	97.61
16	11.62	55.54	9.80	70.65

TABLE 4.—INDIVIDUAL LEAF AND TOTAL PLANT LEAF AREA FOR PLANTS OF CLONAL LINE 146 GROWN UNDER FIVE DIFFERENT PHOTOPERIODS

Photoperiods	Total leaf area of plant in sq. cm.	Number of leaves	Area of individual leaf in sq. cm.
8	378.83	43	8.81
10	392.13	38	10.32
12	406.95	27	15.07
14	606.34	31	19.60
16	423.82	18	23.54

Individual leaf areas were measured on one typical clonal line after approximately one month's exposure to treatment. The data obtained are given in Table 4.

In other words, leaf area increases with increasing length of the day-length-exposure period. This increase and the decrease in number of leaves are reflected in the total leaf area of the plant which reached a maximum, with this clone, under a 14-hour photoperiod.

General growth habit as well as number and size of leaves was, as would be expected, affected by the length of the photoperiod. The plants were arbitrarily classified into the following six groups on the basis of their general appearance:

Erect—An extremely erect compact type.

Widely Erect—Shoots growing more openly than in the erect type.

Ascending—Stems extending first to the side and then curving upward.

Tuft—Petioles of leaves growing erect, forming a cluster.

Prostrate—Stems lying along the ground with only the terminal part raised. This type does not necessarily form a crown.

Prostrate Flat—Crown of short shoots of telescoped buds.

The relative occurrence of the different types is given in Table 5.

TABLE 5.—PERCENTAGE OCCURRENCE OF VARIOUS GROWTH TYPES IN CLONAL LINES OF RED CLOVER GROWN UNDER FIVE DIFFERENT PHOTOPERIODS

Growth type	Percentage of clones of indicated growth type when grown under photoperiods of				
	8 hours	10 hours	12 hours	14 hours	16 hours
Prostrate Flat	90.32	17.14	—	—	—
Prostrate	—	62.86	27.27	—	—
Tuft	9.68	20.00	30.30	16.67	12.50
Ascending	—	—	36.36	30.00	4.17
Widely Erect	—	—	6.07	13.33	12.50
Erect	—	—	—	40.00	70.83

TABLE 6.—DAYS EXPOSURE TO 14- AND 16-HOUR PHOTOPERIODS REQUIRED FOR FLOWERING IN A NUMBER OF CLONAL LINES OF RED CLOVER

Clonal line number	Length of photoperiod	
	14 hours	16 hours
137	145	54
107	138	54
121	143	57
140	141	70
146	150	74
126	147	90
144	—*	90
147	150	91
131	152	92
143	100	116
122	116	125
102	—*	130

* Failed to flower in 156 days.

Flower formation was prevented in all clonal lines when the plants were grown under photoperiods of 12 hours or less. Photoperiods of 14 hours induced flower formation in all but two of the lines, while those of 16 hours induced flowering in all the lines tested. The number of days' exposure to 14- and 16-hour photoperiods required to induce flowering in the various clonal lines is given in Table 6.

Individual clonal lines varied greatly in the number of days required for flowering. Clonal lines 143 and 122 flowered more rapidly under photoperiods of 14 hours than they did under photoperiods of 16 hours. No explanation can be offered for the differences between the number of days required for flowering in this experiment and the one represented in Table 1, where some of the same clonal lines were involved.

Table 7 gives the number of flowers produced per plant in the different clonal lines studied.

TABLE 7.—AVERAGE NUMBER OF FLOWERS PER PLANT IN CLONAL LINES OF RED CLOVER GROWN UNDER 14- AND 16-HOUR PHOTOPERIODS

Length photoperiod in hours	Number of flowers formed in clonal line number											
	146	137	131	144	121	128	140	107	102	143	122	147
16	14.0	12.5	10.0	7.5	6.3	4.7	4.7	4.0	6.0	3.0	5.0	4.0
14	1.0	1.0	1.0	—	1.0	1.0	11.0	2.0	—	26.0	19.0	1.0

Clonal lines 140, 143 and 122 flowered more profusely under the 14-hour photoperiods than they did under the 16-hour photoperiods. The latter two lines are the ones that also flowered earlier under the 14-hour photoperiod.

THE EFFECT OF DAYLIGHT AND LIGHT INTENSITY ON THE GROWTH AND DEVELOPMENT OF RED CLOVER

Light intensity as well as daylength affects the growth and development of plants. The preceding experiments were all conducted in the greenhouse, during the winter, when light intensity was at a minimum. The experiment reported in this section was undertaken out-of-doors, during the summer, in order to determine what effect light intensity had on growth and development and to what extent varying intensity affected the daylength response previously described.

Plants from ten clonal lines propagated by means of crown cuttings were used in this experiment. These were grown under 8-hour daylengths until the beginning of the experiment. The experiment was conducted in a submerged cold frame in which the 6-inch clay pots containing the plants were set in sand. This was done in order to maintain cool conditions around the roots. The plants were exposed to three light intensities. One series of plants was left unshaded; a second series was shaded with a single layer of unbleached cotton and a third series was shaded with a double layer of unbleached cotton. Within each series, half of the plants were exposed to the natural photoperiod of approximately 15 to 16 hours, while the other half were covered with light-proof screens from five p.m. to nine a.m., thus giving them a photoperiod of 8 hours.

PLATE III. The effect of daylength and light intensity on the growth and development of red clover.

A — Clonal line 142 at the end of the first growth period.
B — Clonal line 142 at the end of the second growth period.
C — Clonal line 128 at the end of the first growth period.
D — Clonal line 128 at the end of the second growth period.

From *left to right* in each case:

Heavy shading, long daylength.
Heavy shading, short daylength.
Intermediate shading, long daylength.
Intermediate shading, short daylength.
No shading, long daylength.
No shading, short daylength.

TABLE 8.—GROWTH HABIT RESPONSE OF RED CLOVER PLANTS WHICH WERE SUBJECTED TO DIFFERENT LIGHT INTENSITIES AND DAYLIGHTS FOR TWO SUCCESSIVE PERIODS OF GROWTH

Growth period	Clonal line number	Shading with layers of unbleached cotton					
		None		One layer		Two layers	
		Long day	Short day	Long day	Short day	Long day	Short day
First	121	Erect*	Prostrate	Erect	Widely Erect	Erect	Widely Erect
	139	Erect	Prostrate	Erect	Tuft	Erect	Widely Erect
	159	Erect	Prostrate	Erect	Erect	Erect	Widely Erect
	128	Widely Erect	Prostrate	Widely Erect	Prostrate	Erect	Tuft
	142	Widely Erect	Prostrate	Widely Erect	Widely Erect	Widely Erect	Widely Erect
	147	Erect	Prostrate	Erect	Ascending	Erect	Erect
	157	Erect	Tuft	Erect	Tuft	Erect	Tuft
	143	Erect	Prostrate	Erect	Tuft	Widely Erect	Tuft
	107	Widely Erect	Prostrate	Erect	Erect	Erect	Tuft
	146	Tuft	Prostrate	Erect	Tuft	Erect	Erect
Second	121	Erect	Prostrate Flat	Erect	Prostrate	Prostrate	Prostrate
	139	Erect	Prostrate Flat	Prostrate	Prostrate Flat	Erect	Prostrate
	159	Erect	Prostrate Flat	Prostrate	Tuft	Dead	Prostrate Flat
	128	Erect	Prostrate Flat	Prostrate	Prostrate	Erect	Prostrate Flat
	142	Widely Erect	Tuft	Erect	Prostrate Flat	Prostrate	Prostrate
	147	Erect	Tuft	Prostrate	Prostrate Flat	Prostrate	Prostrate
	137	Erect	Prostrate Flat	Widely Erect	Prostrate Flat	Tuft	Prostrate
	143	Erect	Tuft	Ascending	Tuft	Prostrate	Prostrate
	107	Erect	Prostrate	Erect	Erect	Dead	Tuft
	146	Erect	Prostrate Flat	Ascending	Prostrate Flat	Erect	Prostrate

*Growth types described in connection with Table 5.

Due to the lack of appropriate equipment, no accurate record was kept of the light intensity under the three degrees of shading. It was estimated, however, to be in the neighbourhood of 5,000, 1,500 and 600 foot-candles at the plant surface on a bright day.

Three plants of each clonal line, except for lines 107 and 140 where only one plant was available, were exposed to each of the treatments. At the beginning of the experiment the plants, approximately three months old from time of cutting, were clipped back to four inches in height. Observations were made on them over a period of 6 weeks, after which they were again clipped back to four inches and observations made throughout a second 6-week growth period.

The general appearance of the plants at the end of the first and second 6-week growth periods can be seen from the representative clones shown in Plate III. It is obvious from this plate that plant height decreases with increasing light intensity, but that this effect varies with clonal line. This response occurs independently of the length of photoperiod. Stem elongation is also stimulated by long photoperiods and inhibited by short photoperiods. This response appears to be independent of light intensity. In some clonal lines the combined effect of low light intensity and short photoperiod induced almost the same response as high light intensity and long photoperiod. It appears that the stimulating effect of low light intensity counteracted the inhibiting effect of short photoperiod on stem elongation.

The effect of the various treatments on growth habit is summarized in Table 8. This table shows that the growth habit of the shaded plants exhibited marked differences between the two periods of growth. Differences in the growth habit between the two periods were not as apparent in clones exposed to long photoperiods as they were in those exposed to the 8-hour photoperiod. The stimulating effect of shading on stem elongation was not as apparent during the second growth period as it was during the first period. Most of the clones changed their growth habit from widely erect in the first period to prostrate in the second.

Figure 1 shows the effect of shading on plant height and on number of leaves developed during the two growth periods. Figure 1-A shows that the average plant height for those clones grown under 8-hour photoperiods and shaded with two layers of cotton was considerably less during the second growth period than it was during the first. With increasing light intensity this difference became less evident. For the unshaded clones the difference in plant height between the second and first period of growth seemed to be non-significant. A similar but more marked response to shading, Figure 1-B, occurred in plants grown under long photoperiods. Figure 1-C shows that during the first period of growth the average number of leaves for plants grown under 8-hour photoperiods was increased with decreased light intensity. During the second period of growth, however, the average number of leaves per clone showed a decrease with decreasing light intensity. This difference in the average number of leaves per clone between the two periods of growth was more marked in plants grown under medium and high light intensities. The increased number of leaves during the second period of growth in plants grown under one layer of cheesecloth can be explained

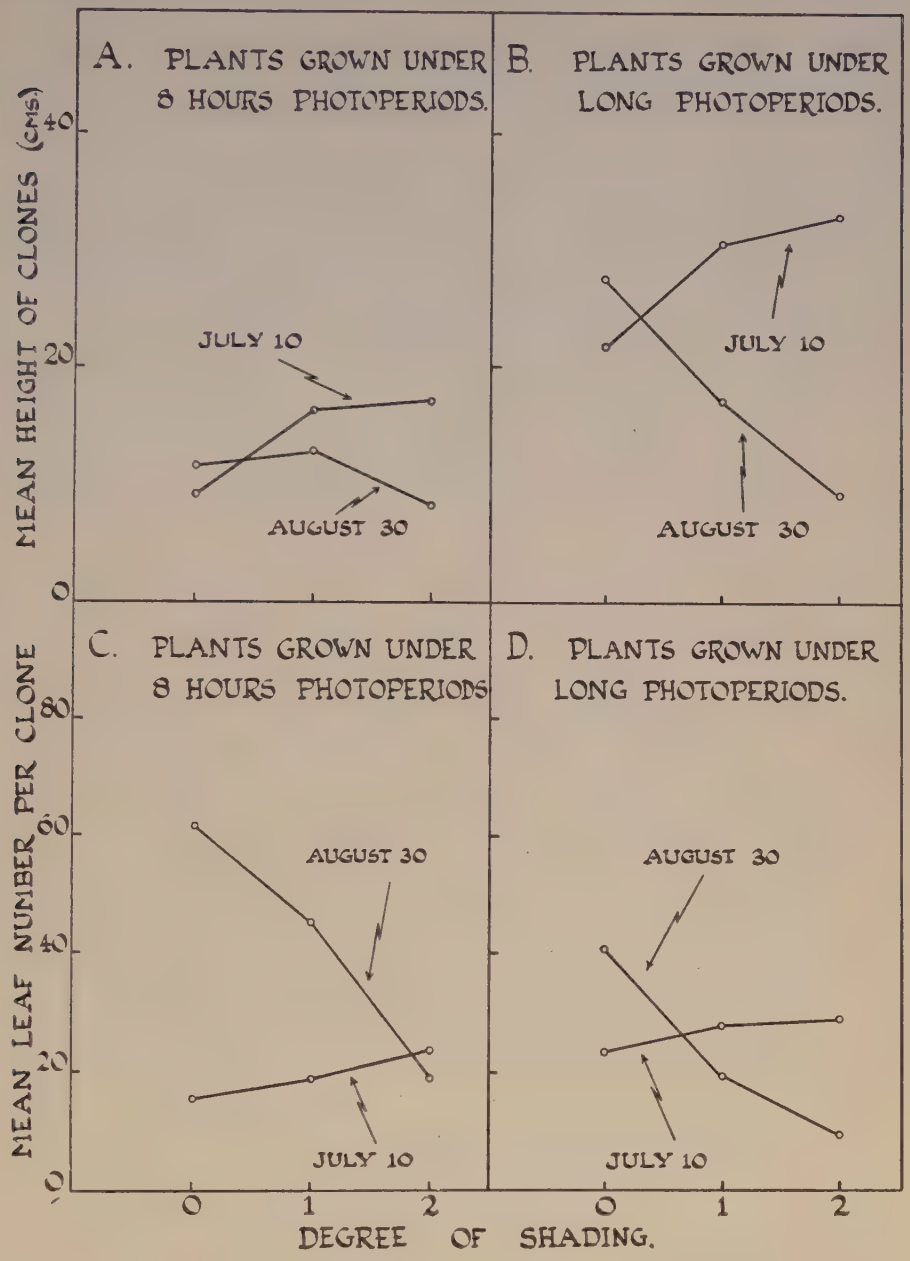


FIGURE 1. Effect of light intensity and daylength on the average plant height and leaf number of plants maintained for two subsequent periods of growth under three degrees of shading and two light intensities.

by their change in growth habit from erect to prostrate. Figure 1-D shows that during the first period of growth the average number of leaves for plants grown under long photoperiods was increased with decreasing light intensity.

Plants grown under 8-hour photoperiods did not form flowers during either period of growth. As previously stated, low light intensity induced shoot formation and elongation during the first growth period of these plants. This stimulating effect of low light intensity on growth was not capable of overcoming the inhibiting effect of short photoperiods on development.

Table 9 shows the effect of shading on flower formation under a long photoperiod. During the first growth period the number of flowers formed per plant appears to increase with increased shading, while the reverse was true during the second growth period. Clonal lines 121, 139 and 159, which flowered under heavy shading during the first growth period, grew very poorly or died during the second growth period.

TABLE 9.—EFFECT OF THREE DEGREES OF SHADING ON THE AVERAGE NUMBER OF FLOWERS FORMED PER PLANT GROWN UNDER LONG PHOTOPERIODS

Clonal line	Number of flowers formed per plant shading with cotton					
	Two layers		One layer		Unshaded	
	July	August	July	August	July	August
121	2.0	—	—	1.3	1.0	2.3
139	2.0	—	1.0	—	1.0	—
159	2.0	—	2.0	—	1.0	3.0
128	—	—	—	—	—	2.0
142	—	—	—	2.0	—	1.3
147	—	—	—	—	—	3.3
137	—	—	—	—	—	4.0
146	—	—	—	—	—	1.0

Heavy shading had an adverse effect on survival as is shown in Table 10. As already indicated, and illustrated in Plate III, this effect varied with clonal line.

TABLE 10.—THE EFFECT OF LENGTH OF DAY AND DEGREE OF SHADING ON THE PERCENTAGE SURVIVAL OF RED CLOVER CLONES

Length of photoperiod	Degree of shading*		
	0	1	2
16 hours	100	100	78
8 hours	100	100	81

* Number of layers of cotton.

DISCUSSION

The results reported on the effect of light on the growth and development of red clover suggest a number of considerations of interest in the development of improved strains of this crop.

Inherent variability in growth habit shows greater expression under conditions of reduced daylength. Two-months-old seedling plants, exposed to daylengths of 8 hours during their second month, showed wide variability and could be selected for ultimate growth habit. Corresponding plants receiving 16-hour daylengths appeared to be very uniform. Clonally propagated plant lines also showed great inter-line variability under 8-hour daylengths. This variability tended to decrease with increasing daylength reaching a minimum under the longest daylength tested (16 hours) in the

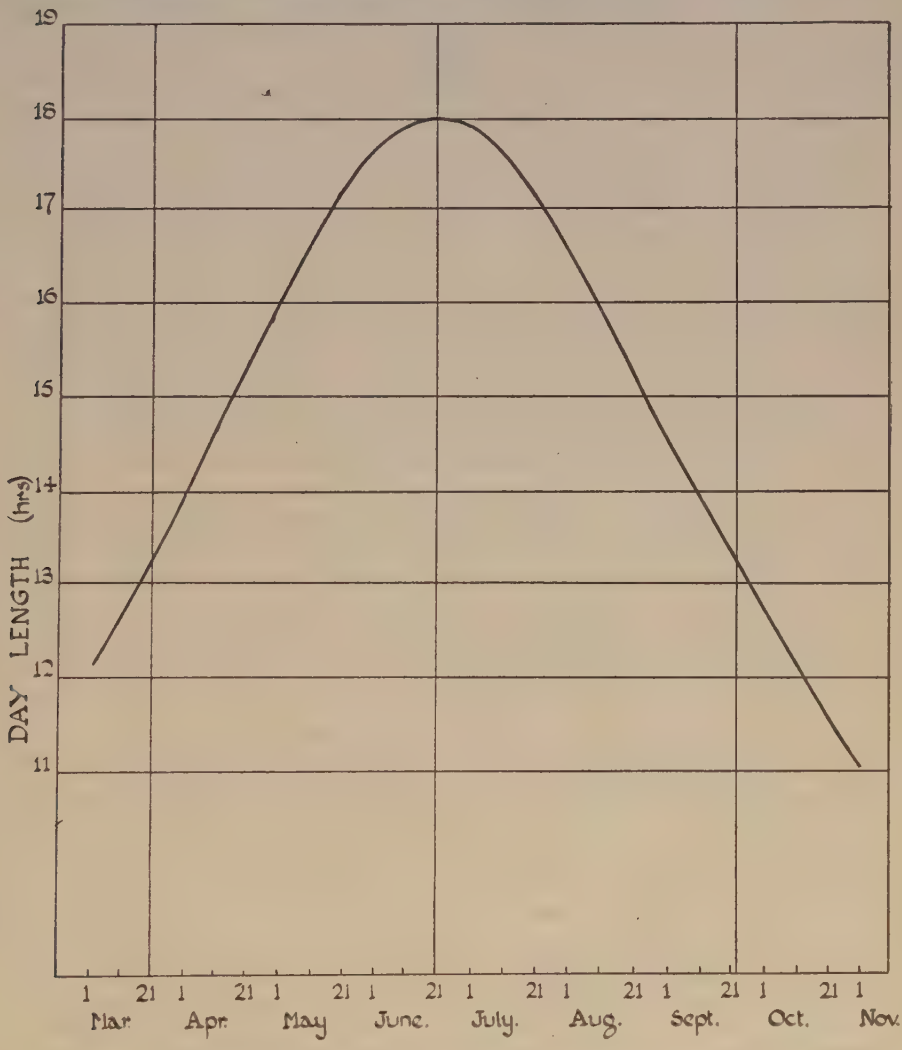


FIGURE 2. Changes in daylength during the growing season at a latitude of 50° N.

preflowering stage. Figure 2, adapted from Post (4), shows the normal daylength prevailing in the latitude of Southern Canada (50° N.) during the normal growing season. It will be noted that the normal daylength prevailing between April 1 and October 1 is far in excess of the minimum 8 hours used in these experiments. Morphological variability would be expected to be at a minimum during the latter half of June and at a maximum in the late fall regrowth following cutting. This is borne out by field observations. Greenhouse selection under conditions of artificially reduced daylength offers a means of speeding up a red clover breeding program.

Flowering is completely inhibited in the Dollard variety of red clover by daylengths of less than 14 hours. A 14-hour photoperiod was found to be the critical one for most of the clonal lines studied but a 16-hour photoperiod was the critical one with two lines. A 16-hour photoperiod was found to induce earlier and more profuse flowering than a 14-hour photoperiod in all clonal lines but two. These were found to flower earlier and more profusely under 14-hour photoperiods. This constitutes definite evidence of variability in daylength response within the variety. Keller and Patterson (3) found that the Emerson variety, grown in the United States, flowered abundantly under photoperiods of 14 hours. Although these authors did not study individual clonal lines their observations would suggest a shorter critical daylength than that required for the Dollard variety. Since this variability exists within the species, it should be possible to select for a daylength response that is optimum for the latitude in which the crop is to be grown. Such selection may already have been inadvertently practised in the Dollard variety. Although this point has not been investigated, the early or double-cut type may consist predominantly of plants able to flower under 14- or 16-hour photoperiods. Under field conditions these plants could flower in June after having received the long days required to bring about flower formation. Once the first cutting is obtained, around the 20th of June, the plants continue growth and again come into flower about the 20th of August. The late or single-cut type, on the other hand, may consist predominantly of plants that required a 16-hour photoperiod. Under field conditions these plants would flower only after being exposed to the long days in June. After cutting they would show only vegetative regrowth because of the shortness of the prevailing photoperiod.

The duration of the photoperiodic stimulation required to induce flowering varies with age of the plant, the older the plant the shorter being the required period of stimulation. It was also found to vary with clonal line. Selection for uniformity in this regard should result in more uniform plant maturity and larger seed yield. Inadequate stimulation often resulted in the production of abortive inflorescences and this might, in practice, be one of the causes of failure to set seed.

Reduced light intensity tends to counteract the effect of reduced daylength on vegetative growth habit. Individual clonal lines differed in their ability to thrive under a wide range of light intensity. Some lines were able to grow well under heavy shading while others under the same conditions failed to survive. This fact would have a definite bearing on

the stand obtained when planting is done with a nurse crop. It provides an explanation for the observation that field stands are often improved by grazing the nurse crop instead of harvesting it in the normal fashion. If planting with a nurse crop is a desirable practice, stands could be improved by breeding for ability to grow well under heavy shading as well as under normal light intensities.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial assistance received from the Quebec Department of Agriculture during the course of this work.

REFERENCES

1. Barrales, H. G., and R. A. Ludwig. The clonal propagation of red clover. Sci. Agr. 32 : 455-462. 1952.
2. Bird, J. N. Early and late types of red clover. Sci. Agr. 28 : 444-453. 1948.
3. Keller, E. R., and N. L. Patterson. Effect of photoperiod on red clover and timothy strains grown in association. Agron. J. 42 : 598-603. 1950.
4. Post, K. Effect of daylength and temperature on growth and flowering of some florist crops. Cornell Agr. Expt. Sta. Bull. 787. 1942.

PRELIMINARY REPORT ON THE EFFECT OF SPRAY FOR CORN BORER CONTROL ON YIELDS OF FIELD CORN[1]

G. F. H. Buckley and C. G. Mortimore[2]

Dominion Experimental Station, Harrow, Ont.

[Received for publication October 20, 1952]

ABSTRACT

Experiments were conducted with field corn in 1950 and 1951 at the Dominion Experimental Station, Harrow, Ontario, to study the effects of one and two sprays of parathion and DDT upon the yields of shelled corn. Five hybrids and one open-pollinated variety of corn were included in this study. Spray applications were made (*a*) when fifty egg masses were present per 100 plants, (*b*) one week later and (*c*) two weeks later.

In 1950 an average increase in yield of 8.9 bushels per acre was obtained with two sprayings of parathion. In 1951 the single applications of DDT gave average increases of 13.9 and 17.1 bushels of corn for first and second dates of spraying respectively, while two sprayings gave an average increase of 16.6 bushels.

The results indicate that one spraying, applied seven to ten days after egg-mass counts reach 50 per 100 plants, is just as effective in increasing yields as two sprayings.

INTRODUCTION

In recent years successful control of the European corn borer has been obtained in sweet corn through the use of some of the newer insecticides. It is only natural that the question should arise as to what effect the control or partial control of the European corn borer would have upon the yield of field corn. With this question in mind, a study was begun in 1950 at the Dominion Experimental Station, Harrow, Ontario, in co-operation with the Dominion Entomological Laboratory, Chatham, Ontario. The latter is responsible for that portion of the study which relates to corn borer population. This progress report is based upon two years' findings and deals only with the effect of spraying upon height of plant, maturity and yield.

Literature concerned with the effect of spraying for corn borer control upon the yield of corn appears to be very limited. Decker and Bigger (1) report that in strip tests on eleven fields they obtained an increase in yield of fourteen bushels per acre on the sprayed strips and a reduction in the average number of corn borers per plant from six to two.

MATERIALS AND METHODS

Five popular commercial hybrids and one open-pollinated variety were chosen for this investigation. Parathion was the insecticide used in 1950 at a concentration of 1.66 pounds of 15 per cent parathion per 100 gallons of water. Because of the highly toxic properties of parathion, in 1951 this spray material was replaced by DDT, an equally effective insecticide against the corn borer. Concentration of this spray was two pounds of 50 per cent wettable DDT per 100 gallons of water. Sufficient spray was applied to wet the plants, particularly the whorls and the leaf axils.

[1] Contribution from the Dominion Experimental Station, Harrow, Ontario.
[2] Senior Agrostologist and Agrostologist, respectively.

In 1950 the following three treatments were laid down:

S1.A—A single spray applied on date set for first application.

S2 —Sprayed twice, first spray applied on above date and another spray two weeks later.

U —Unsprayed.

The date of first application was set as that time when 50 egg masses were present per 100 plants. In 1951 another spray treatment was added: S1.B—a single spray applied one week later than S1.A. The dates on which the sprays were applied are given in Table 1.

TABLE 1.—DATES OF PLANTING AND APPLICATION OF INSECTICIDE IN 1950 AND 1951

Year	Date planted	Dates of spraying*			
		S1.A	S1.B	S2	
				First	Second
1950	June 1	July 7	—	July 7	July 21
1951	May 16	June 27	July 6	June 27	July 11

* First spray applied when corn borer egg masses numbered 50 per 100 plants

A split-plot design of four replications, with spray treatments as the subplots, was selected for this experiment. Plot size was four hills by eleven hills. Only the central two rows of the four-row plots were sprayed, the outside two rows of each plot acting as guards against spray drift.

No attempt was made to obtain complete control of the corn borer. It was felt that with field corn two applications of spray were all that would be practical. Also applied at the above dates corn is not often over three feet tall which eliminates the necessity of a high-clearance sprayer for applying the spray.

RESULTS AND DISCUSSION

The data for 1950 and 1951 are summarized in Table 2.

The sprayed plots had a much better appearance than the unsprayed ones. The former showed much less leaf injury and greatly reduced tassel breakage.

Plant heights were not taken in 1950 as there were no noticeable differences between treatments within a hybrid. In 1951 all sprayed treatments were taller. Nearly 100 per cent of the tassels in the unsprayed plots were broken over thus accentuating the difference. In measuring these plots the tassels were held upright in order to obtain the true height of the plants. The shorter growth in the unsprayed plots was undoubtedly due to a reduction in plant vigour brought about by the excessive leaf and stalk feeding of the corn borers.

In both years the ears harvested from the sprayed plants showed a slightly higher moisture content than those gathered from the unsprayed ones. Possibly the greater damage caused by the borers in the untreated plots produced a degree of premature ripening of the corn plants.

Increases in yield were obtained from spraying in both years but were more pronounced in 1951, the year of heavier borer infestation. In 1950 the yield of S1.A treatment was no better than that from the untreated plots. This was due either to the fact that the spray was applied too early

TABLE 2.—AVERAGE PLANT HEIGHT, MATURITY RATING AND YIELD FOR EACH SPRAY TREATMENT

Treatment	Plant height	Moisture in grain at harvest	Yield*	Increase in yield over check (U)
	in.	%	bu.	bu.
		1950 (Parathion)		
U	—	25.7	74.4	—
S1.A	—	25.9	76.0	1.6
S2	—	26.7	83.3	8.9
L.S.D. at 5 per cent level			3.3	—
		1951 (DDT)		
U	98	19.8	72.7	—
S1.A	104	20.4	86.6	13.9
S1.B	107	20.5	89.8	17.1
S2	108	20.9	89.3	16.6
L.S.D. at 5 per cent level			3.9	—

* Yield of shelled corn per acre at 15 per cent moisture.

or more probably the corn was too short. Decker and Bigger (1) state that there is a low survival of borers hatching on plants that are less than 24 inches tall. Accepting this statement and since the corn was only 15 inches tall when the S1.A spray was applied, the mortality of the borers would be high without spraying and consequently little benefit would result from its use.

The S2 treatment yielded definitely better than either U or S1.A treatments, giving an average increase of 8.9 bushels over the check. Since the S1.A treatment was not effective, the increase in yield from the S2 plots must be due to the second spraying. This was applied when the plants were approximately thirty inches tall. The second spray then would correspond very closely to the S1.B treatment in 1951.

In 1951 the yields of all spray treatments were far superior to the unsprayed. Treatments S1.B and S2 would appear to be better than S1.A although the increases in yield were not significantly greater statistically. The highest yield was obtained from the S1.B treatment. It would appear that this single spray had been applied at a very opportune time and had destroyed most of the corn borer larvae that otherwise would have caused the greatest damage to the corn plants.

From the data obtained to date, it would seem that, in years of average to heavy corn borer infestation, spraying for corn borer control would be a paying proposition. To express the beneficial results of corn borer control on a monetary basis an arbitrary value of $1.60 for a bushel of shelled corn may be assumed. Excluding treatment S1.A in 1950, the range in increased gross returns per acre through spraying is from $14.24 for S2 in 1950 to $27.36 for S1.B in 1951. Since the cost of custom spraying one acre of corn with DDT is between $5.00 and $6.00 outstanding net profits remain.

REFERENCE

1. Decker, G. C., and J. H. Bigger. Spraying and dusting field corn for corn borer control. Univ. of Illinois Circular 642. 1949.

A STUDY OF THE INTER-RELATIONSHIPS OF SEED YIELD, OIL CONTENT, AND OTHER AGRONOMIC CHARACTERS WITH SUNFLOWER INBRED LINES AND THEIR TOP CROSSES

W. A. Russell[1]

[Received for publication October 16, 1952]

ABSTRACT

The data for this study were obtained in 1949 and 1950. The variety Sunrise was used as the top-cross parent. The correlation coefficients between the characters, days to flowering, height of plants, and rust rating of the inbred lines, and the same characters in their top crosses were positive and highly significant in both years, and likewise for head diameter and per cent of lodged plants in 1949. No definite relationships were found between ten plant characters of the inbred lines and their combining ability as expressed by yields of their top crosses. The self-fertility of inbred lines and the degree of hybridization under open pollination had a highly significant correlation coefficient of − 0.5008 in 1949. Oil content and kernel content had a positive, highly significant correlation in the inbred tests for two years and in the 1950 top-cross test. The correlation coefficient between per cent of oil in seed of the inbreds and in seed of their top crosses was positive and significant in 1949 and positive and highly significant in 1950. The multiple correlation of + 0.5315 in 1949 for the relationship between oil content and eight plant characters of inbred lines was significant for N = 54. In 1950, the multiple correlation coefficient for the same relationship was + 0.5730 which was practically equal to the 5 per cent level of significance for N = 45.

INTRODUCTION

Breeding programs to develop varieties of sunflowers improved for seed yield, oil content, and other agronomic features have been in progress in Canada for approximately 15 years. The principal breeding method adopted has been the selection of inbred lines from open pollinated varieties, and more recently from planned crosses, and subsequent recombination of these lines into single crosses and top crosses to restore vigour and increase seed yields. The top-cross hybrid, Advance, was introduced for commercial use in 1946, and now it is practically the only type being used for oil seed production in Canada.

The selection of inbred lines and subsequent testing of these to determine their combining ability in hybrid combinations is an expensive and time-consuming procedure. It would be advantageous to the plant breeder to know if some of the more easily measured agronomic features could be used in selecting lines with high combining ability. Improved strains of sunflowers must contain a high percentage of oil in the seed. The laboratory analysis to determine oil content is costly. Consequently, it would be of value to learn whether or not some other seed characters might be used in the preliminary selection for high oil-bearing types.

This study was designed to determine the relationships between various plant characters of inbred lines and their combining ability as expressed by yields of their top crosses. The experiment was planned also to find out if some easily measurable seed characters could be used in the selection for seed types with high oil content.

[1] Formerly Agricultural Scientist in charge of the corn and sunflower breeding programs, Canada Experimental Farms Service, Morden, Man.; now Assistant Professor, Agronomy Department, Iowa State College of Agriculture, Ames, Iowa.

REVIEW OF LITERATURE

The extent to which selection during the inbreeding program has been effective in the isolation of desirable inbred lines has been studied in maize by several investigators and to a lesser extent in onions and sunflowers. Jones and Mangelsdorf (9) found no single criterion by which high yielding strains of inbred corn could be selected. They emphasized that effective selection must be based upon the performance of the plants after homozygosity has been obtained. Hayes (5) obtained a strong correlation between yield and other characters of inbred lines which are expressions of vigour. Kiesselbach (11) reported that there appeared to be a general relationship between the productivity of inbred lines of maize and their hybrid offspring. Richey and Mayer (14) concluded that, although it is desirable to select lines which are productive in themselves, the final selection must be based on the performance in hybrid combination rather than in the self-fertilized condition. Nilsson-Leissner (12) and Jorgenson and Brewbaker (10) studied the relationship between several characters of selfed lines of maize and their yields in first generation crosses. They concluded that the selection of the most vigorous selfed lines for use in hybrid combinations was the proper procedure for practical plant breeding.

Jenkins (7) conducted a more detailed study of the relationship of inbred lines of corn and the various F_1 crosses between them. Within the inbred lines he obtained positive and significant correlation coefficients between yield and plant height, number of ears per plant, ear length, ear diameter and shelling percentage; and negative and significant correlations between yield and date of silking, shrinkage of harvested ears, chlorophyll grade and ear-shape index. Yield of the F_1 crosses was correlated significantly and positively with the following characters in the parents: Date of tasseling, date of silking, plant height, number of nodes per plant, number of nodes below the ear, number of ears per plant, ear length, ear diameter and yield. There was a significant negative correlation between yield of the F_1 crosses and ear-shape in the parents. The multiple correlation for mean yield of the cross-bred progeny and four characters of the parent lines indicating plant vigour and size was $+ 0.42$. In general, characters indicating vigour and size were correlated positively with yield in the inbred lines and in the crosses.

Johnson and Hayes (8) found that the combining ability of inbred lines of sweet corn was determined in only a small part by the characters studied. In a more extensive study involving 110 field corn inbred lines Hayes and Johnson (6) found that yield of the inbred-variety crosses was correlated significantly with twelve characters which represent vigour in the inbred lines. A multiple correlation between yield of the inbred-variety crosses and these twelve characters gave a coefficient of $+ 0.6660$. This was further evidence which supported the practice of selecting vigorous inbred lines for their seed production and for their combining ability in hybrid combinations.

Aylesworth (1) found highly significant correlations between inbred characters and the same characters in the inbred-variety crosses. Correlations for yields of the inbred-variety crosses and the characters of the inbred lines were positive and significant in the case of date of silking, plant height,

yield, ear length and date of pollen shedding. The multiple correlation of + 0.5940 for the relationship between inbred-variety yields and eleven inbred characters was non-significant.

Ferguson (3) studied the relationship between characters of inbred lines of onions and crosses between these lines and six male-sterile lines. He obtained a highly significant multiple correlation of + 0.6795 between male sterile-inbred cross yields and seven inbred characters representing vigour. The results indicate the importance of selecting for vigorous onion inbreds to obtain those with the greatest combining ability in hybrid combination.

Some studies of this nature with sunflowers have been reported by Canadian investigators. Ross (15) made a study of some morphological characters of sunflowers and their relationship to the yield of seed and oil. He found highly significant and negative correlation coefficients between seed yield and the characters, number of leaves, number of branches, days to blooming and number of heads. There was a highly significant and positive correlation between seed yield and plant height. There was not a significant relationship between yield and the characters, area of leaves and diameter of the main heads. Oil content of the seed and the characters, plant height and seed yield, showed a highly significant and positive correlation. Oil content of the seed did not show significant associations with the characters, area of leaves, number of leaves, diameter of main heads, number of branches, number of days from seeding to blooming and number of heads.

Putt (13) studied the association of sunflower seed yield with four plant characters, days from seeding to maturity, height, stem diameter and head diameter. In his experiment with 188 inbred lines the first season and 60 inbreds the second season he obtained highly significant positive correlations between seed yield and these four plant characters. The multiple correlation between yield and the four plant characters was + 0.821 the first year and + 0.773 the second year. None of these five characters was correlated significantly with oil content of the whole seed. There was a highly significant and positive correlation between per cent oil of the whole seed and per cent kernel of the whole seed. There appeared to be no definite association of oil content with seed size and weight per bushel. The multiple correlation measuring the association between oil content and the characters, days to maturity, plant height, stem diameter, head diameter, seed yield, 1000-seed weight and per cent kernel was + 0.599. Since the partial correlation between oil content and kernel content was + 0.519, most of the variability accounted for in oil content was due to kernel content.

The safflower has a seed type similar to sunflower, except that it is smaller, and may contain approximately the same amount of oil. Claassen *et al.* (2) found highly significant correlations of − 0.77 and −0.81 in two studies involving oil content and hull content. The correlation between oil content and seed size was + 0.22 which was highly significant. They investigated two methods of estimating oil content of the seed sample. The first method consisted of external examination of the seed combined with cutting through a few seeds of each sample. The second method

consisted of thoroughly crushing several grams of seed from each sample and visually comparing the crushed material with three standards of known oil content. Correlations of + 0.93 and +0.90 were obtained between the estimated and actual oil percentage for the two methods, respectively. Approximately 75 per cent of the samples were estimated within 2 per cent of the actual oil percentage. These results show that the estimation of oil percentage is of value in eliminating low oil selections before any chemical analysis are made in breeding material.

MATERIALS AND METHODS

The sunflower breeding program commenced at the Experimental Station, Morden, Manitoba, in 1943. Selection and inbreeding were started in open pollinated varieties which were being grown in the area, and in single crosses and multiple crosses obtained from the Forage Crops Laboratory in Saskatoon, Saskatchewan. Introductions received from various sources were available also for further selection and selfing. In 1947 several of the lines which had been developed were sufficiently uniform in plant and seed type to permit bulking of the seed within each strain.

In 1948, 84 inbred lines were planted in the nursery for selfed seed increase. These lines were seeded also in an isolated top-cross block with Sunrise being used as the male parent. Isolation obtained was one-quarter mile, which would not be complete but considered satisfactory for this study. Sunrise was planted in the alternate rows and in two rows completely surrounding the crossing block. The inbreds were seeded in the remaining rows, approximately ten feet of row being used for each. All of the Sunrise was drilled on one day and the inbreds on dates following so that they should have bloomed coincidently with the male parent. Any lines which did not flower at the same time as the male parent were removed. Cross pollination could occur between the various lines as well as from Sunrise to the inbred strains. However, since Sunrise is an excellent pollen producer, it was believed most of the crossing on to the selections would be by pollen of this variety.

TABLE 1.—SOURCE, NUMBER OF LINES FROM EACH SOURCE AND GENERATIONS SELFED FOR 62 INBRED LINES OF SUNFLOWERS

Cross number	Source, cross or variety	Number of lines	Number of generations selfed
1	952-30 × 149	1	4
3	154 × Sunrise	1	4
4	952-30 × Sunrise	2	4
5	Sunrise × 952-56	10	4
10	(S-37-388 × Sunrise) (S-37-402 × S-488)	8	4
11	(S-37-388 × Sunrise) (S-37-402 × S-488)	15	4
12	(S-37-388 × Sunrise) (S-37-402 × S-488)	6	4
15	S-488 × S-37-388	1	4
17	S-37-388 × Sunrise (Advance)	4	4
19	S-37-402 × S-37-388	3	4
22	(Sunrise × S-488) (S-37-402 × S-37-388)	3	4
	Mennonite	1	4
	Mennonite	3	8+
	Introductions	4	4+

Sufficient quantities of seed for both the top crosses and inbreds resulted in 62 lines being available for study. The sources of the inbred lines are shown in Table 1.

Inbreds S-37-388 and S-37-402 were developed from the Mennonite variety. The selections 952-30, 149, 154, 952-56, and S-488 were introductions and the original sources are unknown. Inbred S-488 appears to be closely related to Sunrise.

The experiment was set up in 1949 as two separate, adjacent tests, one being the top crosses and the other, the inbreds. Sunrise and Advance were included as checks in each trial. A partially balanced lattice design with six replications was used in each test. The plots consisted of two rows, 20 feet long with 36 inches between rows and plots. Seeding was done at approximately double the normal rate and stands were thinned later so that plants were eight to ten inches apart in the row.

Studies on Yield and Plant Characters

Notes were taken in all replications for date of flowering, plant height, head diameter, root lodging, broken plants, rust infection, number of plants harvested and seed yield in both inbred and top-cross tests. Additional notes were made in the inbred trial on leaf area, stalk diameter, general vigour, and date of maturity. The plant character notes for the hybrid test were made relative to the hybrid plants since most plots contained some progeny resulting from sib- or self-pollination in the top-cross block. Hybrid plants generally showed considerable more vigour than the plants from sib- or self-pollinated seed.

Rust readings were made on a rating basis in which the following scale was used:

0 — Immune (no lines available for this study).
1 — Pustules on the lower leaves, none on the upper leaves.
2 — Many pustules on the lower leaves, some on the upper leaves.
3 — Many pustules on all leaves, but no defoliation.
4 — Many pustules on all leaves, defoliation had occurred on the lower half of the plant.
5 — Defoliation almost or totally complete.

The inbreds were rated for general vigour on a scale of one to five wherein one was for least vigour and five was for greatest vigour. Maturity dates were not taken for the hybrids because they ripened slowly and many of them were killed by frost before showing signs of plant maturity. Final weights for seed yields were obtained after all samples had been allowed to become uniformly dry. For the top-cross trial, only the seed weights for hybrids were used.

This experiment was repeated in 1950 using 44 of the inbreds studied in 1949 and three new ones. Those lines not continued the second season were: five from cross 5; one from cross 10; eight from cross 11; one from cross 12; and two introductions. The origins of those added were: one from cross 10; one from cross 12; and one from Mennonite. In this season, the experiment was set up in a split plot design so that adjacent to each top-cross plot was the female parent. The main plots were arranged in a P6 lattice with six replications. This permitted easier selection of the hybrid plants in the top-cross plots. The same notes were taken as in

1949 with the exception of dates of maturity for the inbreds. Ripening was very slow and frost occurred before many of the lines showed signs of plant maturity.

Analysis of Data

The 1949 top-cross test included several plots which had few or no hybrid plants or in which it was difficult to distinguish between hybrid and sib- or self-pollinated progeny. As a result, data for eight inbreds and their top crosses were not used in the study of plant characters and yields. The data for Sunrise and Advance were not included in any of the statistical analyses.

Separate analyses of variance to determine significance of differences were run on all character measurements in the inbred tests, and on all character measurements in the top-cross trials with the exceptions of per cent of lodged and broken plants in 1950. These data were not analysed, due to the occurrence of a large number of zero readings. Data in percentages were transformed to sin $^2\theta$ before being analysed.

There was considerable variation between plots in the number of plants harvested. A covariance analysis was used for the inbred trials and the 1950 top-cross trial, and a curvilinear regression analysis was used for the 1949 top-cross test to obtain a more accurate estimate of yields (4) (16).

Simple correlation coefficients were calculated for all inter-relationships of the adjusted top-cross yields with ten inbred characters in 1949 and with eight inbred characters in 1950. The multiple correlation coefficients were computed to determine the total effect of these inbred characters on top-cross yields. Simple correlation coefficients were calculated, also, between characters of the inbred lines and the same characters of the hybrids.

The inbreds studied consisted of one group which was derived from crosses having Sunrise as one parent and a second group which had no genetic relationship to this variety. The mean values for the different characters were divided into the two groups on the basis of this relationship. Simple correlation coefficients were calculated between top-cross yields and inbred characters in the related and unrelated groups for each of the two years.

The inbred lines tested in 1949 were seeded in the 1949 breeding nursery for further increase of selfed seed. At threshing, the number of seeds set per head under controlled self pollination was determined for five normally developed heads per line. The number of plants harvested and weight of seed per plot were known for the inbred yield trial. For each inbred in the test the weight per 1000 seeds was obtained. An approximate estimate of the average number of seeds set per head under open pollination could be calculated from these data. A comparison of the average number of seeds set under self pollination and under open pollination would give a relative estimate of the per cent of seed setting under controlled selfing for each line. The simple correlation coefficient between per cent of seed set on selfing and degree of hybridization under open pollination was determined.

Oil and Seed Studies

A second phase of this experiment involved a study of the relationship between the characters, oil content, kernel content or shelling percentage, weight per 1000 seeds, and weight per bushel. In 1949, a composite seed sample from all six replications was taken for each inbred and top cross whereas, in 1950, composite samples were taken from the first three and last three replications.

Two methods of estimating oil content were used on the 1950 inbred test. The first method consisted of rating the seed for oil content on the basis of seed appearance and structure as determined by crushing with the thumb nail. For the second method, a small sample was coarsely ground in a hand grist mill and the per cent of oil estimated by the appearance and texture. In both cases, three standards of known oil content, low, medium and high, were used for making comparisons.

The oil data for 1949 and data for all seed characters, including the methods of estimating per cent of oil, for 1950 were analysed statistically to determine significance of differences. Correlation studies were made for oil content and the various seed characters. The relationship between inbred and top-cross oil content was studied. Oil content estimated by method two was correlated with the actual determinations. Simple correlation coefficients were computed for inbred oil content and ten plant characters of inbred lines in 1949, and eight plant characters of inbred lines in 1950.

EXPERIMENTAL RESULTS

1. Studies on Yields and Plant Characters of Inbreds and Top Crosses

Significance of Differences between Inbreds and between Top Crosses

The F values for the variety variance compared with error variance for the various character measurements are given in Table 2. The F

TABLE 2.—F VALUES FOR VARIETY VARIANCE COMPARED WITH ERROR VARIANCE FOR THE AGRONOMIC CHARACTER MEASUREMENTS OF 54 INBRED LINES AND THEIR TOP CROSSES TESTED IN 1949, AND 47 INBRED LINES AND THEIR TOP CROSSES TESTED IN 1950

Character	F values, 1949		F values, 1950	
	Inbreds	Top crosses	Inbreds	Top crosses
Yield	15.10*	8.75*	6.90*	6.64*
Days to flowering	41.11*	33.95*	94.16*	16.18*
Days to maturity	21.67*	—	—	—
Height of plant	44.03*	14.28*	46.92*	8.64*
Diameter of head	4.72*	3.55*	7.63*	1.21
Diameter of stalk	42.91*	—	10.26*	—
Area of leaves	14.44*	—	25.22*	—
Per cent lodged plants	6.45*	4.77*	3.87*	—
Per cent broken plants	12.20*	3.73*	2.56*	—
Rust rating	14.51*	4.07*	12.24*	2.29*
Vigour rating	11.65*	—	18.34*	—

* Exceeds the one per cent level of significance.

TABLE 3.—FREQUENCY DISTRIBUTION FOR AGRONOMIC CHARACTERS OF (a) 54 INBRED LINES TESTED IN 1949, (b) 54 TOP CROSSES TESTED IN 1949, (c) 47 INBRED LINES TESTED IN 1950, AND (d) 47 TOP CROSSES TESTED IN 1950. CLASS CENTRES ARE SEPARATED BY THE LEAST SIGNIFICANT DIFFERENCE AT THE FIVE PER CENT POINT, ARRANGED ON EACH SIDE OF THE 0 CLASS WHICH CONTAINS ALL PROGENIES WHICH DID NOT DIFFER SIGNIFICANTLY FROM ADVANCE

Character	Test	Class centres															L.S.D. 5 per cent point	Mean of Advance	Mean of experiment	Range of means
		−10	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	+1	+2	+3	+4				
Yield—pounds per acre	a				4	16	15	10	6	1	2						157.2	1849	922.0	537–1537
	b							1	8	28	10	7					175.1	1543	1165.7	813–1688
	c				12	23	6	4	2								147.3	1194	264.0	98–663
	d								3	17	18	9					248.0	1157	708.7	221–1204
Days to flower	a						4	3	11	10	7	11	4	1	3		1.81	77.2	74.2	66.5–83.1
	b						1	1	10	16	3	15	3	3	2		1.70	79.5	77.2	69.5–86.3
	c			1	4	3	5	5	8	2	8	4	1	2	2	2	1.65	82.7	78.3	68.2–90.5
	d									4	16	21	2	2	2		2.55	83.0	81.8	76.3–91.2
Days to mature	a						1	1	6	6	12	21	6	1			2.38	131.8	129.1	119.7–136.7
Height of plant—inches	a	1	0	2	4	6	8	11	6	4	9	1	2				2.52	51.8	41.0	26.0–55.0
	b							1	3	10	10	27	2	1			3.30	55.0	51.9	40.5–62.
	c	1	1	3	5	6	6	7	7	7	1	3					2.78	60.5	46.7	30.7–58.
	d								1	6	10	30					3.85	61.2	58.3	47.4–64.9
Diameter of head—centimeters	a								24	16	12	2					1.34	18.6	15.0	13.3–17.8
	b									3	18	33					1.20	18.9	17.8	15.7–19.3
	c						1	11	14	17	14	0					1.20	18.2	14.2	11.2–16.8
	d										2	45					1.25	16.2	15.8	14.4–17.0

TABLE 3.—FREQUENCY DISTRIBUTION FOR AGRONOMIC CHARACTERS OF (a) 54 INBRED LINES TESTED IN 1949, (b) 54 TOP CROSSES TESTED IN 1949, (c) 47 INBRED LINES TESTED IN 1950, AND (d) 47 TOP CROSSES TESTED IN 1950. CLASS CENTRES ARE SEPARATED BY THE LEAST SIGNIFICANT DIFFERENCE AT THE FIVE PER CENT POINT, ARRANGED ON EACH SIDE OF THE 0 CLASS WHICH CONTAINS ALL PROGENIES WHICH DID NOT DIFFER SIGNIFICANTLY FROM ADVANCE—*Con.*

Character	Test	Class centres														L.S.D. 5 per cent point	Mean of Advance	Mean of experiment	Range of means
		−10	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	+1	+2	+3	+4				
Diameter of stalk—centimeters	a					2	2	11	13	11	11	4					0.13	2.6	2.2	1.8–2.6
	c						2	8	13	17	6	1					0.19	2.8	2.2	1.8–2.7
Leaf area square—decimeters	a				1	9	12	15	8	6	3						7.21	76.8	43.8	25.0–65.9
	c							7	6	7	12	12	2	1			7.26	55.8	43.0	22.8–73.6
Per cent lodged plants*	a										2	43	6	2	1		6.75	17.4	20.1	7.0–39.4
	b										4	44	6				6.32	11.2	12.3	2.2–23.0
	c											31	13	2	1		9.77	0.0	8.8	0.0–36.4
	d																—	0.0	9.3	0.0–20.2
Per cent broken plants*	a										27	22	2	2	0	1	8.63	17.0	12.0	0.0–53.6
	b										6	45	2	1			9.48	12.0	10.1	0.0–31.8
	c									1	28	18					8.98	21.6	12.2	2.5–25.2
	d																—	9.1	13.8	0.0–33.8
Rust rating 0-5	a								3	6	4	18	17	14	1	1	0.36	3.1	3.2	1.7–4.8
	b									4	9	41					0.59	3.5	3.2	2.2–4.0
	c									1	3	28	10	5			0.53	2.5	2.7	1.2–4.0
	d										1	45	1				1.20	3.0	3.0	1.5–4.5
Vigour rating 1-5	a					1	6	14	17	12	1	3					0.53	5.0	3.1	1.7–4.8
	c					1	4	10	11	8	9	4					0.54	5.0	3.3	1.7–5.0

* Values in per cent transferred to $\sin^2\theta$.

values for all characters except head diameter of top crosses in 1950 exceed the one per cent level of significance. It will be noted that for both years the F values were lower in the top crosses tested than in the inbred lines.

Frequency Distribution for Agronomic Characters Studied

Frequency distributions are shown in Table 3 for the agronomic characters studied in the inbred and top-cross tests for each year. Direct comparisons of the frequency distributions for a character are not valid because of the difference in size of the L.S.D. for the various tests.

The yields of the inbreds were decidedly lower in 1950 than in 1949. The season of 1949 was much more favourable for production of this crop. Also, in 1950 many of the inbreds were infested by larvae of *Phalonia hospes* Wlshm. which destroyed the kernels. The range of yields of the inbreds was large in both seasons and all inbreds have produced significantly below Advance. The hybrid yields, likewise, were lower in 1950, but the difference was not as great as for the inbreds. None of the top crosses yielded significantly higher than Advance, but seven crosses in 1949 and nine in 1950 were placed in the zero class on the basis of the L.S.D.

The data in Table 3 show the behaviour of the inbred lines and top crosses in comparison with Advance for the other plant characters, also. Of particular interest are the ranges in days to flower, plant height, leaf area, rust rating, and vigour rating for the inbreds. Vigour ratings of the inbreds give a numerical picture of the growth vigour as compared to Advance.

Correlation Studies of Characters of the Inbreds and the Top Crosses

Simple correlation coefficients were calculated to show the degree of association between six plant characters of the inbred lines and the same characters of the top crosses. The r values are given in Table 4.

TABLE 4.—CORRELATION COEFFICIENTS BETWEEN THE MEAN VALUES FOR THE AGRONOMIC CHARACTERS OF INBRED LINES AND THE MEAN VALUES FOR THE SAME CHARACTERS IN THEIR TOP CROSSES IN 1949 AND 1950[1]

Character	Correlation coefficient	
	1949	1950
Yield	+ 0.1848	+ 0.0073
Days to flowering	+ 0.9066**	+ 0.8626**
Height of plant	+ 0.8166**	+ 0.6451**
Diameter of head	+ 0.3977**	—
Per cent lodged plants	+ 0.5068**	—
Rust rating	+ 0.5049**	+ 0.4916**

[1] = 54 in 1949 and 47 in 1950.
** Exceeds the one per cent level of significance.

The r values for yields of inbreds and top crosses did not reach the level for significance in either 1949 or 1950. All the remaining r values have exceeded the one per cent point of significance. The correlation coefficients for days to flowering in 1949 and 1950 and for height of plant in 1949 were high.

TABLE 5.—CORRELATION COEFFICIENTS FOR ALL INTER-RELATIONSHIPS BETWEEN 10 AGRONOMIC CHARACTERS OF 54 INBRED LINES, AND THE RELATIONSHIP OF EACH CHARACTER WITH MEAN YIELD OF THE TOP CROSSES—1949

——	Yield of top crosses	B	C	D	E	F	G	H	I	J
B. Yield of inbred	+ 0.1848									
C. Leaf area	+ 0.0259	+ 0.6863**								
D. Plant height	+ 0.2055	+ 0.5994**	+ 0.7278**							
E. Days to flowering	+ 0.0984	+ 0.6290**	+ 0.7646**	+ 0.7436**						
F. Days to maturity	− 0.1462	+ 0.4185**	+ 0.4772**	+ 0.4418**	+ 0.5051**					
G. Stalk diameter	− 0.0784	+ 0.4087**	+ 0.5549**	+ 0.4346**	+ 0.5088**	+ 0.5118**				
H. Head diameter	+ 0.1404	+ 0.5479**	+ 0.7143**	+ 0.6154**	+ 0.6033**	+ 0.4731**	+ 0.5605**			
I. Rust rating	− 0.0194	− 0.2284	− 0.1779	− 0.2817*	− 0.1806	− 0.5432**	− 0.2831*	− 0.1404		
J. Vigour rating	+ 0.0598	+ 0.7383**	+ 0.8768**	+ 0.6820**	+ 0.8173**	+ 0.5677**	+ 0.5012**	+ 0.7527**	− 0.2053	
K. Per cent lodged plants	+ 0.0758	+ 0.1426	+ 0.3761**	+ 0.3155**	+ 0.1640	+ 0.2209	+ 0.2444	+ 0.3734**	− 0.1628	+ 0.3661**

* Significant at the five per cent level.
** Significant at the one per cent level.

TABLE 6.—CORRELATION COEFFICIENTS FOR ALL INTER-RELATIONSHIPS BETWEEN EIGHT AGRONOMIC CHARACTERS OF 47 INBRED LINES, AND THE RELATIONSHIP OF EACH INBRED CHARACTER WITH MEAN YIELD OF THE TOP CROSSES—1950

	Yield of top crosses	B	C	D	E	G	H	I
B. Yield of inbred	+ 0.0073							
C. Leaf area	− 0.0565	+ 0.4275**						
D. Plant height	− 0.2733	+ 0.2548	+ 0.6432**					
E. Days to flowering	− 0.1911	+ 0.1520	+ 0.7298**	+ 0.7272**				
G. Stalk diameter	− 0.5384**	− 0.0021	+ 0.4367**	+ 0.5741**	+ 0.5308**			
H. Head diameter	+ 0.1322	+ 0.3514*	+ 0.7237**	+ 0.3350*	+ 0.3559*	+ 0.3091*		
I. Rust rating	+ 0.0451	+ 0.1959	+ 0.2216	+ 0.1456	+ 0.0641	− 0.1003	+ 0.1596	
J. Vigour rating	− 0.1931	+ 0.2920*	+ 0.8822**	+ 0.6219**	+ 0.7119**	+ 0.5385**	+ 0.7099**	+ 0.2291

* Significant at the five per cent level.
** Significant at the one per cent level.

The simple correlation coefficients were calculated from the 1949 data for all inter-relationships between ten agronomic characters of 54 inbred lines, and the relationship of each inbred character with the mean yields of the top crosses. The r values are given in Table 5. The same calculations were made with the 1950 data for eight characters of 47 inbred lines and the top-cross yields. The r values are shown in Table 6.

Simple correlation coefficients for the 1949 data were positive and highly significant for all inter-relationships of the inbred characters, yield, leaf area, plant height, days to flowering, days to maturity, stalk diameter, head diameter and vigour rating. Per cent of lodged plants had a positive and significant correlation with plant height, and positive and highly significant correlation coefficients with leaf area, head diameter and vigour rating. Rust rating was negatively correlated with all characters, the coefficients being significant with plant height and stalk diameter and highly significant with days to maturity.

In the 1950 data, Table 6, all inter-relationships of the inbred characters, leaf area, plant height, days to flowering, stalk diameter, head diameter and vigour rating, had positive and significant, or highly significant, correlation coefficients. Inbred yield had positive and significant correlations with head diameter and vigour rating, and a positive and highly significant correlation with leaf area. Rust rating was not significantly correlated with any of the inbred characters.

The correlation coefficient showing the degree of association between top-cross yields and stalk diameters of inbreds was negative and highly significant for the 1950 data. For 1949 and 1950 data, none of the other correlation coefficients involving top-cross yields and inbred characters was significant. The multiple correlation coefficients were computed to show the total effect of the inbred characters on top-cross yields for each season. The coefficient of + 0.4712 for the 1949 test was not significant, whereas the value of + 0.6761 for the 1950 test was highly significant.

The inbred lines were divided into two groups, the first being those not genetically related to Sunrise and the second being those developed from crosses which had Sunrise as one parent. For each group, simple

TABLE 7.—CORRELATION COEFFICIENTS BETWEEN THE MEAN VALUES FOR SEVEN CHARACTERS OF 11 INBRED LINES NOT GENETICALLY RELATED TO SUNRISE AND THE MEAN VALUES FOR YIELDS OF THEIR TOP CROSSES, AND FOR SEVEN CHARACTERS OF 45 INBRED LINES RELATED TO SUNRISE AND THE MEAN VALUES FOR YIELDS OF THEIR TOP CROSSES, 1949 DATA

Characters of inbreds	r Values	
	Unrelated	Related
Yield	+ 0.3162	+ 0.1456
Leaf area	− 0.4444	+ 0.0669
Height of plant	− 0.1839	+ 0.2758
Days to flower	− 0.0695	− 0.0015
Diameter of stalk	− 0.4144	+ 0.2502
Diameter of head	− 0.2628	+ 0.1402
Vigour rating	− 0.0690	+ 0.0010

TABLE 8.—CORRELATION COEFFICIENTS BETWEEN THE MEAN VALUES FOR SEVEN CHARACTERS OF 12 INBRED LINES NOT GENETICALLY RELATED TO SUNRISE AND THE MEAN VALUES FOR YIELDS OF THEIR TOP CROSSES, AND FOR SEVEN CHARACTERS OF 35 INBRED LINES RELATED TO SUNRISE AND THE MEAN VALUES FOR YIELDS OF THEIR TOP CROSSES, 1950 DATA

Characters of inbreds	r Values	
	Unrelated	Related
Yield	+ 0.5513	− 0.0751
Leaf area	+ 0.0671	− 0.2891
Height of plant	− 0.0582	− 0.3887*
Days to flower	− 0.0468	− 0.3521*
Diameter of stalk	− 0.3967	− 0.3079
Diameter of head	− 0.3444	− 0.0874
Vigour rating	− 0.0908	− 0.3335*

* Exceeds the five per cent level of significance.

correlation coefficients were calculated between the mean values for yield of top crosses and the mean values for seven characters of the inbred lines. The r values are given in Tables 7 and 8.

The 1949 data had no significant correlation coefficients between characters of the inbreds and top-cross yields for either the unrelated or related groups. In the 1950 test for the group related to Sunrise, top-cross yields and each of the inbred characters, height of plant, days to flower and vigour rating had a negative and significant correlation coefficient. In the unrelated group, the correlation coefficient between inbred yields and top-cross yields was just below the five per cent level for significance.

Inbred yields for 1949 and 1950 had a simple correlation coefficient of + 0.3655 which was significant at the five per cent level. The top-cross yields for 1949 and 1950 had a correlation coefficient of + 0.4160 which was highly significant. The simple correlation coefficient of − 0.2147 between inbred yields in 1949 and top-cross yields in 1950 was not significant.

Relationship between Self Fertility and Cross Fertility of Inbred Lines

A study was made of the relationship between per cent of seed set under selfing bags in 1949 and the per cent of cross pollination in the 1948 crossing block as determined in the 1949 top-cross test. The simple correlation for these two characters was − 0.5008 which is highly significant. The mean values for per cent of hybrid plants in the 1949 test ranged from 4.6 to 76.7. The relative mean values for per cent of seed set under selfing bags were 4.2 to 155.3.

2. Studies on the Relationship between Oil Content and Seed and Plant Characters

Significance of Differences between Inbreds and between Top Crosses for Per Cent of Oil and Other Seed Characters

The F values computed by comparing the variety mean square to the error mean square are shown in Table 9. The F value for kernel content of the 1950 top crosses exceeded the five per cent point for significance whereas the F values for all other measurements exceeded the one per cent level.

TABLE 9.—F VALUES FOR VARIETY VARIANCE COMPARED WITH ERROR VARIANCE FOR THE SEED CHARACTER MEASUREMENTS OF 62 INBRED LINES AND 36 TOP CROSSES TESTED IN 1949, AND 45 INBRED LINES AND 44 TOP CROSSES TESTED IN 1950

Character	F Values			
	1949		1950	
	Inbreds	Top crosses	Inbreds	Top crosses
Oil content	56.92**	138.87**	4.30**	2.83**
Kernel content	—	—	6.66**	2.06*
Weight per 1000 seeds	—	—	10.90**	8.32**
Weight per bushel	—	—	9.94**	12.05**

* Exceeds the five per cent level of significance.
** Exceeds the one per cent level of significance.

Frequency Distributions for Seed Characters Studied in Inbreds and Top Crosses

Frequency distributions are shown in Table 10 for the seed characters studied for the inbred and top-cross tests for each year. Direct comparisons cannot be made between frequency distributions because of variations in size of the least significant differences.

The data in Table 10 present an interesting comparison between Advance and the inbred lines and top crosses for the various seed characters. In 1949, two inbreds were significantly higher than Advance for oil content and in 1950, one inbred was significantly higher. Four top crosses exceeded Advance for oil content in 1949 by more than the significant margin. The range of means for each test of seed characters was large, and was greater for the inbred lines than for the top crosses in each year.

Correlation Coefficients of Oil Content with Other Seed Characters in the Inbreds and Top Crosses

The r values for the four tests involving the seed characters are given in Table 11.

The inbred tests had positive and highly significant correlation coefficients between per cent of oil and kernel content, but the other associations were less definite. The per cent of oil and kernel content in the top crosses was positive and highly significant in 1950.

The multiple correlation coefficients were computed to show the total relationship between oil content and the seed characters, kernel content, weight per 1000 seeds, and weight per bushel for the four tests. The values obtained for the inbreds were + 0.6772 in 1949 and + 0.8144 in 1950, and for the top crosses they were + 0.2992 in 1949 and + 0.4614 in 1950. The coefficients for the inbred tests were highly significant, the value for the 1950 top-cross test exceeded the five per cent level of significance, and the value for the 1949 top-cross test was not significant.

The correlation coefficients were calculated to show inter-relationships between mean values for per cent of oil in the inbreds and top crosses in 1949 and 1950. The r values, together with the number of observations, are given in Table 12.

TABLE 10.—FREQUENCY DISTRIBUTION FOR OIL CONTENT AND THREE SEED CHARACTERS OF (a) 62 INBRED LINES TESTED IN 1949, (b) 36 TOP CROSSES TESTED IN 1949, (c) 45 INBRED LINES TESTED IN 1950, AND (d) 44 TOP CROSSES TESTED IN 1950. CLASS CENTRES ARE SEPARATED BY THE LEAST SIGNIFICANT DIFFERENCE AT THE FIVE PER CENT POINT ARRANGED ON EACH SIDE OF THE 0 CLASS WHICH CONTAINS ALL PROGENIES WHICH DID NOT DIFFER SIGNIFICANTLY FROM ADVANCE

Character	Test	−14	−11	−10	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	+1	+2	+3	+4	+5	5	L.S.D. per cent point	Mean of Advance	Mean of experiment	Range of means
Oil content—per cent	a				2	3	4	5	7	15	6	4	5	9	2						1.59	35.6	29.2	20.9–37.6
	b	1	1	1	2	0	2	4	3	0	3	3	9	3	1	1	0	1	1		0.65	36.2	34.0	26.7–39.8
	c											5	18	21	1						4.53	30.9	25.9	19.7–36.4
	d											5	20	19							2.92	32.0	28.6	23.4–31.8
Kernel content—per cent	a																				—	63.0	59.4	40–70
	b																				—	59.0	59.3	51–67
	c											2	12	28	3						5.16	58.5	55.7	46.5–65.0
	d												2	42							3.92	57.0	56.9	52.5–60.5
Weight per 1000 seeds—grams	a																				—	68.5	52.6	37.2–72.0
	b																				—	71.2	65.3	53.6–78.0
	c									11	18	11	2	3							6.58	71.6	50.7	40.2–77.2
	d											1	16	24	2	1					7.15	71.1	66.6	53.8–90.6
Weight per bushel—pounds	a																				—	27.0	27.0	20.5–32.0
	b																				—	29.0	28.4	26.0–32.5
	c										2	5	14	22	2						2.01	27.2	25.1	20.9–29.4
	d											2	15	21	4	2					1.65	27.0	26.2	23.4–30.8

TABLE 11.—CORRELATION COEFFICIENTS FOR ALL INTER-RELATIONSHIPS BETWEEN MEAN VALUES FOR OIL CONTENT AND THREE SEED CHARACTERS OF 62 INBREDS AND 36 TOP CROSSES TESTED IN 1949, AND 45 INBREDS AND 44 TOP CROSSES TESTED IN 1950

	Inbreds—1949		
	Oil content	B	C
B. Kernel content	+ 0.6083*	—	—
C. Weight per 1000 seeds	− 0.4009*	− 0.3991*	—
D. Weight per bushel	+ 0.4579*	+ 0.3675*	− 0.1948
	Inbreds—1950		
	Oil content	B	C
B. Kernel content	+ 0.7906*	—	—
C. Weight per 1000 seeds	− 0.2586	− 0.1235	—
D. Weight per bushel	− 0.0958	− 0.0068	− 0.0057
	Top crosses—1949		
	Oil content	B	C
B. Kernel content	+ 0.2199	—	—
C. Weight per 1000 seeds	− 0.1580	+ 0.1457	—
D. Weight per bushel	− 0.0481	+ 0.2155	+ 0.1994
	Top crosses—1950		
	Oil content	B	C
B. Kernel content	+ 0.4464*	—	—
C. Weight per 1000 seeds	− 0.2096	− 0.2363	—
D. Weight per bushel	− 0.0033	+ 0.1127	+ 0.0015

* Exceeds the one per cent level of significance.

TABLE 12.—CORRELATION COEFFICIENTS SHOWING INTER-RELATIONSHIPS BETWEEN MEAN VALUES FOR OIL CONTENT IN INBREDS AND TOP CROSSES IN 1949 AND 1950

	r	N
Inbreds 1949 with inbreds 1950	+ 0.6918**	43
Inbreds 1949 with top crosses 1949	+ 0.4062*	36
Inbreds 1949 with top crosses 1950	+ 0.3676*	41
Inbreds 1950 with top crosses 1950	+ 0.4914**	42
Top crosses 1949 with top crosses 1950	+ 0.4955**	28

* Exceeds the five per cent level of significance.
** Exceeds the one per cent level of significance.

Two correlation coefficients have exceeded the five per cent level of significance and three have surpassed the one per cent level.

Methods for Estimating Per Cent of Oil in Inbred Seed

Oil contents of the inbred seed samples from the 1950 test were estimated by appearance of the whole seed and seed structure and by appearance and texture of a coarsely ground sample. An analysis of variance was made on the data for each method. The F value for differences in the estimated values for per cent of oil was not significant in the first method and was highly significant in the second.

Table 13 shows the agreement of mean estimated oil content, based on appearance and texture of a coarsely ground sample, with mean oil content determined by ether extraction. The simple correlation coefficient between oil content as estimated by method two and actual oil content was + 0.6129 which was highly significant.

TABLE 13.—AGREEMENT OF MEAN ESTIMATED OIL CONTENT, BASED ON APPEARANCE AND TEXTURE OF A COARSELY GROUND SAMPLE, WITH MEAN OIL CONTENT DETERMINED BY CHEMICAL ANALYSIS

Error of estimated oil analysis—per cent	Number of estimations	Percentage of total
0 to 1	19	42.2
1 to 2	7	15.6
2 to 3	10	22.2
3 to 4	2	4.4
4 to 5	2	4.4
5 to 6	2	4.4
6 to 7	2	4.4
7 to 8	1	2.2

Figure 1 shows four coarsely ground seed samples ranging from 20.6 to 36.3 per cent of oil as determined by ether extraction. The estimated oil contents for these samples were 18, 26, 34 and 32 per cent.

TABLE 14.—CORRELATION COEFFICIENTS BETWEEN THE MEAN VALUES FOR OIL CONTENT AND AGRONOMIC CHARACTERS OF INBRED LINES IN 1949 AND 1950

Character of inbred	Correlation coefficient	
	1949	1950
Yield	+ 0.2503	+ 0.0668
Days to flowering	+ 0.2442	+ 0.5377**
Days to maturity	+ 0.2287	—
Height of plant	+ 0.4426**	+ 0.3093*
Diameter of head	+ 0.1662	+ 0.2285
Diameter of stalk	+ 0.2534	+ 0.1802
Leaf area	+ 0.2494	+ 0.4347**
Per cent lodged plants	+ 0.1729	—
Rust rating	− 0.2736*	− 0.0084
Vigour rating	+ 0.2605	+ 0.3890**

N=54 in 1949 and 45 in 1950.
* Exceeds the five per cent level of significance.
** Exceeds the one per cent level of significance.

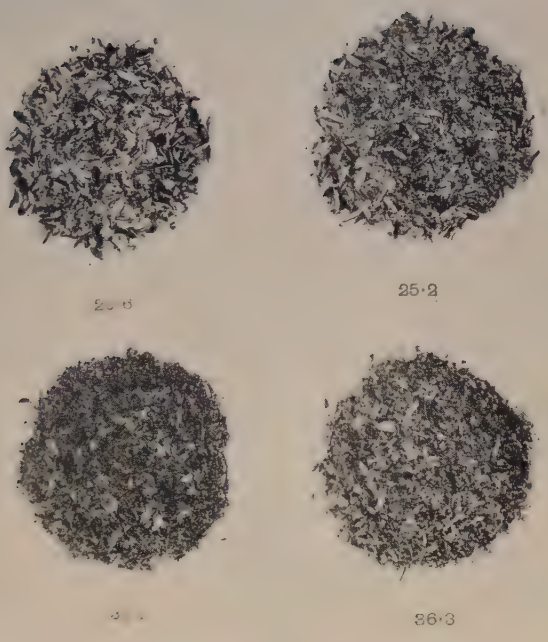


FIGURE 1. Samples of coarsely ground sunflower seed which vary in per cent of oil from 20.6 to 36.3 per cent.

Correlation Coefficients of Oil Content with Plant Characters of Inbreds

Simple correlation coefficients were calculated between the mean values for per cent of oil in the inbreds and ten agronomic characters in 1949, and eight agronomic characters in 1950. The r values are given in Table 14.

Oil content and plant height had a positive and highly significant correlation coefficient the first year, and positive and significant correlation coefficient the second year. In the second season, per cent of oil showed a positive and highly significant correlation with the characters, days to flowering, leaf area and vigour rating. There was a negative and significant correlation between oil content and rust rating in 1949.

The multiple correlation coefficient indicating the effect of eight inbred characters, days to maturity and per cent of lodged plants excluded, on per cent of oil in the inbreds in 1949 was + 0.5315 which was significant for N = 54. The value for the 1950 data was + 0.5730 which was practically equal to the five per cent level of significance for N = 45.

DISCUSSION

The results for this experiment were obtained in two seasons which were entirely different from the standpoint of growing conditions. In 1949 seeding was done during the second week of May whereas in 1950 a cold, wet spring delayed planting until the last week of May. The growing season of 1949 was featured by normal temperatures and adequate rainfall until the end of July and then hot, dry weather during August. In 1950 the entire season was generally wet and temperatures were below normal. Consequently, some variation between results for the two seasons would be anticipated.

The male parent, or tester, used in this study was Sunrise which originated as an inbred line. It had been propagated by open pollination using space isolation to maintain purity for at least six generations prior to the commencement of this experiment. Further inbreeding within Sunrise was begun in 1944. The lines which have been developed vary considerably for such characters as height, days to flowering, leafiness, plant strength and rust reaction. There are significant differences between them in combining ability as has been determined by studying them in hybrid combinations. Therefore, it may be expected that this variety should give a satisfactory measure of general combining ability when used as a tester parent. The inbred lines tested in this experiment differed significantly in combining ability as was shown by the F values in Table 2 and the frequency distributions in Table 3. None of the hybrids was significantly superior to Advance in yield in either season but a number of them were placed in the same class as this check. One top cross, which was classed with Advance in both seasons, was superior to it in respect to yield of the female parents. This is an important consideration from the standpoint of hybrid seed production.

The inbreds themselves showed highly significant differences for all characters studied. These results suggest that there is good opportunity for improvement of inbred lines in respect to the more important features such as seed yield, per cent of oil, plant strength, general vigour and rust resistance.

When the inbreds were studied as one group, no significant correlation coefficients were found between top-cross yields and ten inbred characters studied in 1949. In 1950 with eight inbred characters, only stalk diameter was significantly correlated with top-cross yields, and in this case the coefficient was negative and highly significant. Three of the higher yielding top crosses in 1950 had female parents with mean stalk diameters significantly below the experiment mean, and three of the lower yielding top crosses had female parents with stalk diameters significantly above the experiment mean. These six top crosses and their female parents could have been responsible for most of the correlation between top-cross yields and stalk diameters of the inbreds.

The multiple correlation of + 0.4712 in 1949 which is an expression of the association between top-cross yields and ten inbred characters indicates that only 19 per cent of the variability in the hybrid yields was due to this association. This correlation was non-significant. The highly significant multiple correlation coefficient of + 0.6761 in 1950 for the relationship of eight inbred characters and top-cross yields indicates that 46 per cent of the variability in hybrid yields has been accounted for by the inbred characters. This value is approximately the same as was obtained by Hayes and Johnson (6) in field corn studies and by Ferguson (3) in his work with onions. However, in this experiment over two-thirds of the variability of top-cross yields accounted for has been due to the association with stalk diameter.

When the inbred lines were divided into two groups on the basis of genetic relationship to the tester parent, no significant r values were found in 1949 between top-cross yields and eight inbred characters in either group. In 1950 with the group genetically related to Sunrise, there were negative and significant correlations between top-cross yields and the inbred characters, height of plant, days to flowering and vigour rating. With the unrelated group, the correlation between inbred and top-cross yields was positive and closely approached the five per cent level of significance.

The absence of any definite relationships between inbred characters and combining ability of the inbreds as expressed by yield in hybrid combinations is at variance with most results obtained in studies with corn. This behaviour may have been due in part to the genetic relationship between Sunrise and many of the lines. However, there appears to be some other factor involved since there were no significant r values between top-cross yields and characters of those lines unrelated to Sunrise. This variety may contain a large number of factors responsible for high combining ability and many of these have a dominant, or nearly dominant, behaviour. Consequently, the only lines which showed superior combining ability in the test crosses were those which possessed genes complementary to the genes already present in Sunrise. In other words, in this experiment Sunrise has given a measure of specific combining ability and not general combining ability for the lines studied.

If sunflower single or top crosses are to be used on a commercial scale it is essential from the economic aspect of first generation hybrid seed production that the parental lines are relatively high yielders. Therefore, in the development of potential parent lines it is important to know which

plant characters contribute most to inbred seed yields. In 1949, there were positive and highly significant correlation coefficients between inbred yields and the vigour characters, leaf area, height of plant, head diameter, stalk diameter and vigour rating. The correlations between yield of inbreds and the characters, days to flower and days to mature were positive and highly significant, indicating that selection for earlier maturity may result in lower yielding lines. These results are in agreement with those reported by Putt (13). Ross (15) found no correlation between seed yield and the characters, leaf area and head diameter, and a negative and significant correlation between seed yield and days to flower. Some of the material which he used possessed the branching habit and this may be responsible for some differences in results. The association of inbred seed yields with plant characters was not as strong in 1950. Yields in this test were seriously affected by partial or total destruction of the kernel by larvae of the insect, *Phalonia hospes* Wlshm. Furthermore, some lines were shattered by birds before the heads could be harvested.

Sunflower rust, *Puccinia helianthi*, may reduce seed yields considerably in some seasons. Consequently, it is important that new lines being developed should show tolerance or resistance to this fungus. Rust rating and days to maturity had a negative and highly significant correlation coefficient in 1949. This relationship would be expected because the most susceptible lines were defoliated and forced into pre-mature ripening.

The extent to which inbred characters other than seed yield are expressed in their hybrid progeny is indicated by the correlation coefficients between characters of inbred lines and the same characters in their top crosses. The correlations involving days to flower and plant height are particularly high.

Sunflower inbred lines differ widely in the ability to produce seed under selfing bags. There was a negative and highly significant correlation coefficient obtained between the per cent of seed set under selfing bags and degree of hybridization. Although this result is based on only one season's data, it suggests that the more self-sterile lines may be the better female parents. However, whether or not the highly self-fertile lines may be potential male parents cannot be concluded from these results.

Sunflowers are being grown in Canada for the high quality edible oil contained in the seed. Consequently, oil content of seed is equally as important as total seed yield in the development of improved hybrids or varieties. The F values for variety variance compared to error variance indicate highly significant differences between inbreds and top crosses for per cent of oil in the seed. One line which was inbred and selected from Advance contained significantly more oil than its parent in both seasons. It also was significantly higher than either of the Advance parents, Sunrise and S-37-388. Another sister selection was equal to Advance in 1949 and 3.5 per cent higher in 1950. This suggests that oil content is determined by factors which are partially dominant and complementary in their reaction. Advance hybrid is not much superior to Sunrise in per cent of oil, nor to S-37-388 when the seed development of this inbred has not been affected by rust. Inbreeding and selection in Advance has produced an inbred line which carries, in the homozygous state, factors from both

Sunrise and S-37-388. This indicates the possibility of further increasing oil content in sunflower seed by recurrent selection. Sprague and Brimhall (17) have reported on the successful use of this method of increasing the oil content in field corn.

The positive and highly significant correlations between per cent of oil and kernel content of the seed in 1949 and 1950 indicates a strong relationship between these characters. There were less definite associations between oil content and the features, weight per 1000 seeds and weight per bushel. The correlation coefficient between per cent of oil and weight per 1000 seeds was negative both seasons and highly significant in 1949. This suggests the tendency for the smaller seed types to be higher in oil content. The multiple correlation coefficients calculated with oil content as the dependent variable and kernel content, seed size and weight per bushel as the independent variables, show that 46 per cent of the variability in oil content in 1949 and 66 per cent in 1950 was due to the association with these seed characters. Kernel content was the most important of these three characters in this relationship to oil content. Putt (13) found a strong correlation between per cent of oil and kernel content but established no definite relationship of oil content with seed size and weight per bushel.

Estimating the per cent of oil on the basis of whole seed appearance did not appear satisfactory in this experiment. Claassen *et al.* (2) used this method with safflower seed, which is similar in type to the sunflower seed, and obtained a correlation of + 0.93. The second method which based the estimates on the appearance and texture of a coarsely ground seed sample appeared promising as a means of selecting the seed types with higher oil content. Only 11.2 per cent of the estimated values deviated from the chemical determination by more than the L.S.D. which was found in the analysis of variance for the data based on ether extractions. Apparent differences were small between samples which contained more than 30 per cent oil. Some errors in estimating oil content could be due to variations in colour of the samples.

It may be concluded from this study on seed characters that where the range in per cent of oil is large preliminary selection may be done on the basis of kernel content and appearance and texture of a coarsely ground sample. This would decrease the expense involved in determinations of oil content by ether extraction. However, where difference between seed samples are smaller, as in the case of the top crosses, estimated values probably would not be reliable and chemical analyses would be necessary.

The positive and significant correlation coefficients between per cent of oil in seed of inbreds and of their top crosses is an indication of the extent to which this feature of an inbred line is expressed in its hybrid progeny. This substantiates the necessity of selecting for oil content as well as plant characters in the development of inbred lines.

The simple correlation coefficients suggest an association between inbred oil content and each of the inbred plant characters, days to flower, height of plant, leaf area, vigour rating and rust rating. The high positive correlation between per cent of oil and days to flower in 1950 may have been due partly to damage by larvae of *Phalonia hospes* Wlshm. This insect attacked the earlier lines more severely than the later ones.

Frequently, only a portion of the kernel would be destroyed and such seeds remained in the sample. Consequently, samples with considerable insect damaged seed would be lower in oil content. It is interesting to note the positive correlation coefficients between per cent of oil and the vigour characters although only four of these were significant in the two years. Putt (13) found no significant relationship between per cent of oil and the plant characters, days to mature, height, stalk diameter, head diameter and seed yield. Ross (15) obtained positive and highly significant correlations between per cent of oil and the characters, plant height and seed yield, but no significant relationship for oil content with leaf area, head diameter and days to full bloom.

On the basis of this experiment it appears that Sunrise may not be a good tester for general combining ability. The possibility of using other varieties or of developing a synthetic for this purpose should be investigated. Polycross progeny testing may be satisfactory for determining general combining ability on new selections. Caution with this method would be necessary, however, because in a polycross block a few lines which are good pollen producers could be the male parents for the majority of the crosses. So far in the breeding program the most promising inbred lines have originated from only two sources, Mennonite and Sunrise varieties. It would be desirable to investigate other sources more intensively in order to increase the genetic diversity of the breeding stocks.

ACKNOWLEDGMENTS

The writer wishes to express his sincere appreciation to H. K. Hayes, former Chief of the Division of Agronomy and Plant Genetics, University of Minnesota, who suggested the problem; to E. H. Rinke and H. L. Thomas, Professors of Agronomy and Plant Genetics, University of Minnesota, for their advice regarding the conduct of the study and the preparation of the original manuscript; to T. M. Stevenson, Chief of the Division of Forage Plants, Ottawa, for his suggestions in the preparation of this paper; and to E. D. Putt who arranged for the oil analyses at the Cooperative Vegetable Oils, Limited, Altona, Manitoba.

REFERENCES

1. Aylesworth, J. W. The relationship of maize characters as expressed in inbreds and inbred-variety crosses. M.S. thesis, Univ. of Minnesota. March, 1948.
2. Claassen, C.E.,W.G. Ekdahl, and G. M. Severson. The estimation of oil percentage in safflower seed and the association of oil percentage with hull and nitrogen percentages, seed size, and degree of spininess of the plant. Agron. J. 42 : 503-508. 1950.
3. Ferguson, A. C. A study of the relationship between characters of inbred lines of onions and their crosses. Ph.D. thesis, Univ. of Minnesota. May, 1951.
4. Goulden, C. H. Methods of statistical analysis. John Wiley & Sons, Inc., New York. 1939.
5. Hayes, H. K. Present-day problems of corn breeding. J. Amer. Soc. Agron. 18 : 344-363. 1926.
6. Hayes, H. K., and I. J. Johnson. The breeding of improved selfed lines of corn. J. Amer. Soc. Agron. 31 : 710-724. 1939.
7. Jenkins, M. T. Correlation studies with inbred and crossbred strains of maize. J. Agr. Res. 39 : 677-721. 1929.
8. Johnson, I. J., and H. K. Hayes. The combining ability of inbred lines of Golden Bantam Sweet Corn. J. Amer. Soc. Agron. 28 : 246-252. 1936.

REFERENCES—Concluded.

9. Jones, D. F., and P. C. Mangelsdorf. The improvement of naturally cross-pollinated plants by selection in self-fertilized lines. I. The production of inbred strains of corn. Conn. Agr. Exp. Sta. Bull. 266. 1925.
10. Jorgenson, L., and H. E. Brewbaker. A comparison of selfed lines of corn and first generation crosses between them. J. Amer. Soc. Agron. 19 : 819-830. 1927.
11. Kiesselbach, T. A. Corn investigations. Nebr. Agr. Exp. Sta. Res. Bull. 20. 1922.
12. Nilsson-Leissner, Gunnar. Relation of selfed strains of corn to F_1 crosses between them. J. Amer. Soc. Agron. 19 : 440-454. 1927.
13. Putt, E. D. Association of seed yield and oil content with other characters in the sunflower. Sci. Agr. 23 : 377-383. 1943.
14. Richey, F. D., and L. S. Mayer. The productiveness of successive generations of self-fertilized lines of corn and of crosses between them. U.S.D.A.Bull. 1354. 1925.
15. Ross, A. M. Some morphological characters of *Helianthus annuus* L. and their relationship to the yield of seed and oil. Sci. Agr. 19 : 372-379. 1939.
16. Snedecor, G. W. Statistical methods. 4th ed. The Iowa State College Press, Ames, Iowa. 1946.
17. Sprague, G. F., and B. Brimhall. Relative effectiveness of two systems of selection for oil content of the corn kernel. Agron. J. 42 : 83-88. 1950.